

**JOINT INVENTOR**

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William K. Merkel

**APPLICATION FOR  
UNITED STATES LETTERS PATENT**

**S P E C I F I C A T I O N**

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**TO ALL WHOM IT MAY CONCERN:**

Be it known that we, Ilya Raskin, a citizen of the United States of America, residing at 48 Alexandria Drive, Manalapan, 07726, in the State of New Jersey, and Alexander Poulev, 168 Exeter Street, Highland Park, New Jersey, 08904, a citizen of the United States of America, have invented a new and useful Elicited Plant Products, of which the following is a specification.

## ELICITED PLANT PRODUCTS

This application claims priority to U.S. Patent Application Serial No. 09/130,185, filed August, 6, 1998, and U.S. Patent Application Serial No. 09/203,772, filed June 23, 1998, which is a continuation-in-part application of U.S. Patent Application Serial No. 09/067,836, filed April 28, 1998, which claims priority to U.S. Provisional Application Nos. 60/045,220 and 60/050,441, filed on April 30, 1997 and June 27, 1997, respectively. Each of the above-mentioned provisional and nonprovisional U.S. Patent Applications are herein expressly incorporated by reference.

## BACKGROUND OF THE INVENTION

Plants have long been recognized as providing a potential source of chemical compounds or more commonly products, known as phytochemicals. A wide variety of compounds of commercial interest, including those having pharmaceutical or therapeutic activity, have been discovered to be plant products. In general, such compounds have been recovered either as a crude extract or as purified compounds, which requires the use of complex extraction and purification procedures. While many therapeutically active compounds have been isolated from extracts of plant roots, methods of screening the plants or plant parts for therapeutically active compounds and isolation of compounds demonstrating such activity from exudates have not been fully investigated.

One source of the chemical compounds or products are the leaf surfaces of the plant. The leaf surfaces of higher plants, in particular, are covered with non-cellular cuticular materials, which are non-living tissue that is heterogeneous in chemical composition. These materials can include lipids, wax, and cutin (biopolymer composing fatty and hydroxy fatty acids), as well as proteins and many secondary metabolites associated with leaf surface or present in hairs or trichomes covering leaf surfaces. These cuticular compounds can be assessed or removed by rapid immersion of intact leaves in organic solvents or by running the solvents over the leaf surface (Martin and Batt, 1958; Purdy and Truter, 1963; S. Fernandes et al., 1964). Generally these techniques can avoid contamination by substances within the leaf making the process, which is significantly different from a total tissue extraction. The amounts of cuticular compounds present on the leaf surfaces of different species are variable, but normally lie in the range 0.01-0.5 mg/cm<sup>2</sup>. Cuticular compounds are usually more frequently obtained from the lower leaf surface rather than the upper leaf surface. Waxes and

other cuticular compounds typically are deposited in early stage of leaf growth and continue to deposit throughout the period of leaf expansion (Richmond and Martin, 1959; Baker et al., 1963; Baker et al., 1968). In additional leaf waxes are known to inhibit spore germination of pathogenic fungi (Hafiz, 1952; Topps and Wain, 1957; van Velson 1957; Komo, Akitsma, 1960).

In addition to leaves, plant roots also continuously produce and secrete a characteristically unique set of compounds into their immediate environment (rhizosphere). It has been suggested that up to 10% of photosynthetically fixed carbon is secreted from the roots in the form of biologically active compounds (Shepherd et al 1993; Johansson et al. 1993). Despite the interest and investigation of these such phenomenon, the systematic study of compounds present in root exudates of diverse plant species and their therapeutic activity has not been undertaken. In addition, certain compounds present in root exudates have been shown to play an important role in several biological processes including activation of the Rhizobium genes responsible for the nodulation process (Peters et al 1986; Peters et al. 1988) and, possibly, for vesicular-arbuscular mycorrhiza (VAM) colonization (Tsai and Phillips 1991). Strigol, a germination stimulant for the parasitic plant *Striga asitica*, has been found in the root exudates of many cereals (Siame et al 1993). Moreover, root-secreted compounds called phytosiderophores may be involved in the acquisition of essential plant nutrients from soils (Cakmak et al 1994) and in defense against such toxic metals as aluminum (de la Fuente et al 1997).

For example, genistein and daidzein are isoflavonoids present in a number of plants which have been recognized as having anti-cancer activity. However, such chemicals currently are available only to the consumer in the form of soybean flours and other crude soybean products. Compounds have been recovered from roots and leafs and used either as a crude root exudate extract or as purified compounds which require the use of complex extraction and purification procedures. Other plant chemicals are available in purified forms; however, they are generally recovered only after a costly and laborious tissue extraction procedure. Accordingly, although there is some information about plant or plant parts producing compounds having therapeutic activity, very little literature, if any, is available regarding the systematic application of chemicals or agents to induce or improve the production of therapeutic compounds, compositions, extracts or exudates in plants. More particularly, there remains a need for a systematic method of inducing or improving the

production of chemical compounds or compositions demonstrating antibacterial, antifungal, and anticancer activity.

In particular, such methods do not identify novel agents having therapeutic activity or provide methods of reproducing compounds, extracts, or compositions having therapeutic activity in a consistent manner.

### SUMMARY OF THE INVENTION

The present invention is directed to a method of eliciting chemical compounds in plants. Although a wide variety of elicitors is disclosed, the chemical compound acetic acid is particularly contemplated for inducing or improving compounds or compositions demonstrating anti-microbial (i.e., anti-bacterial or anti-fungal) and/or anti-cancer activity. The plants typically are subjected to treatment and/or conditions to induce (i.e., to initiate or increase) production of such compounds in the plant. Such treatment includes maintaining a living plant or plant portion in contact with water, while alive, in order to induce or improve production of one or more compounds demonstrating therapeutic activity. The living plant or plant portion is subjected to treatment and/or conditions to induce and/or increase production of one or more compounds in the roots of the plant in particular. Such compounds are recovered from the water for example by the process of extracting or exudation. The water may or may not include other ingredients, such as nutrients or elicitors.

The process of the present invention may be employed for commercial production of desired compounds. The commercial production of such compounds can be accomplished in an aqueous medium containing an elicitor. The elicitor can be any elicitor, including for example, chemical elicitors such as acetic acid, which has demonstrated promising activity in inducing and improving compounds having therapeutic activity.

Roots of the plant can be harvested before extraction. The compounds produced or increased in quantity as a result of the treatment can be recovered from the harvested roots and then screened for potential therapeutic activity. The plant and plant parts can be screened for potential compounds of interest by recovering and isolating such compounds from solvent extract of leaves or roots of the plant.

The plants or plant parts of the invention can be specifically grown or maintained for the purpose of recovering compounds therefrom. A chemical compound library generated from the recovered compounds may be used for screening for a desired compound or activity.

In this respect, plants or plant parts which are specifically grown or maintained for the purpose of recovering compounds therefrom are contacted with water while alive in order to recover a variety of compounds, particularly for potential screening. The plants or plant parts are preferably contacted with an elicitor to increase the amount and/or diversity of compounds which can be recovered in the root exudate. The chemical compound library may be used for screening for a desired compound or activity.

In a preferred embodiment, the plant is grown hydroponically or aeroponically. In a particularly preferred embodiment, the roots are harvested in a manner such that the plant remains alive and can grow new roots for future harvesting and recovery of additional compounds.

The invention also provides a method of identifying an agent exuded from or onto a plant surface having therapeutic activity. The method comprises: (a) removing cuticular material located on the surface of a leaf of the plant, for example by contacting the leaf surface with a solvent, thereby resulting in a solvent solution; (b) assaying the solvent solution for the identification of agents of the cuticular material which have therapeutic activity; and (c) analyzing the solvent solution so as to identify the agent which has the therapeutic activity.

The plants which are used in the invention may be any one of a wide variety of plants and may be sexually or vegetatively propagated plants as is further described herein. In particular, plants suitable for use in the invention, such as use in the method for eliciting a compound having therapeutic activity as described below, include: *Atropa belladonna*, *Erythrina flabelliformis*, *Ipomoea tricolor*, *Erythrina crista*, *Celosia cristata*, *Gallium spurium*, *Laurus nobilis*, *Vitis labrusca*, *Gratiola officinalis*, *Symphitum officinalis*, *Hosta fortunei*, *Cassia hebecarpa*, *Thalictrum flavum*, *Scutellaria altissima*, *Portulacca oleracea*, *Scutellaria certicola*, *Physalis* sp., *Geum fauriei*, *Gentiana tibetica*, *Linum hirsutum*, *Aconitum napellus*, *Podophyllum emodii*, *Thymus cretaceus*, *Carlina acaulis*, *Chamaecrista fasciculata*, *Pinus pinea*, *Peganum harmala*, *Tamarindus indica*, *Carica papaya*, *Cistus incanus*, *Capparis spinosa*, *Cupressus lusitanica*, *Diospyros kaki*, *Eryngium campestre*, *Aesculus woerlitzensis*, *Aesculus hippocastanum*, *Cupressus sempervirens*, *Celtis occidentalis*, *Polygonum cuspidatum*, *Elaeagnus angustifolia*, *Elaeagnus commutata*, *Gentiana macrophylla*, *Brassica rapa*, *Sesbania exaltata*, *Sesbania speciosa*, *Spartina potentiflora*, *Brassica juncea*, *Helianthus annuus*, *Poinsettia* sp., *Pelargonium zonale*, *Synapsis* sp., *Leontopodium alpinum*, *Lupinus*

luteus, *Buxus microphylla* var. *japonica*, *Liatris spicata*, *Primula japonica*, *Betula nigra*, *Filipendula vulgaris*, *Lobelia siphilitica*, *Grevillea robusta*, *Reseda luteola*, *Gentiana littoralis*, *Campanula carpatica*, *Ageratum conizoides*, *Psidium guajava*, *Ailanthus altissima*, *Hydrocotyle asiatica*, *Brugmansia suaveolens*, *Thymus pulegioides*, *Thymus lema-barona*, *Thymus serpyllum* (wild), *Gaultheria procumbens*, *Thymus carnosus*, *Thymus thracicus*, *Calycanthus floridus*, *Zingiber officinalis*, *Lamium dulcis*, *Thymus praecox* "arcticus", *Thymus speciosa*, *Thymus pseudolamginosus*, *Thymus vulgaris*, *Ficus religiosa*, *Forsythia suspensa*, *Chelidonium majus*, *Thymus wooly*, *Thymus portugalense*, *Nicotiana tabacum*, *Thymus cytriodorus* "aureus", *Cactus officinalis*, *Lablab purpurea*, *Juglans regia*, *Actinidia chinensis*, *Hemerocallis* sp., *Betula pendula*, *Gardenia jasminoides*, *Taxodium distichum*, *Magnolia loebherii*, *Crataegus praegophyrum*, *Larix decidua*, *Thuja orientalis*, *Thuja occidentalis*, *Cupressocyparis leylandii*, *Pseudotsuga menziesii*, *Abies firma*, *Parthenocissus quinquefolia*, *Allium cernuum*, *Juniperus* "blue pacific", *Taraxacum officinalis*, *Yucca* sp., *Tsuga canadensis*, *Ilex aquifolium*, *Ilex cornuta*, *Taxus hicksii*, *Taxus media*, *Metasequoia glyptostroboides*, *Pinus bungiana*, *Buxus sempervirens*, *Stewartia koreana*, *Prunus* sp., *Betula dahurica*, *Plantago minor*, *Acer palmatum*, *Acer campestre*, *Cotinus coggygia*, *Quercus robur*, *Acer truncatum*, *Achyranthes bidentata*, *Allium japonicum*, *Carum capsicum*, *Agastache mexicana*, *Prunella vulgaris*, *Tagetes minuta*, *Nepeta cataria*, *Ratibida columnaris*, *Aster novae-angliae*, *Myrica cerifera*, *Pittosporum tobira*, *Plantago major*, *Pinus sylvestris*, *Acorus canadensis*, *Pieris japonica*, *Pinus strobus*, *Trifolium pratense*, *Prunus serotina*, *Datura stramonium*, *Geranium maculatum*, *Hydrocotyle asiatica*, *Astragalus sinicus*, *Centaurea maculata*, *Ruschia indurata*, *Myrthus communis*, *Platanus occidentalis*, *Licium barbatum*, *Lavandula officinalis*, *Grevillea robusta*, *Hypophaë rhamnoides*, *Filipendula ulmaria*, *Betula pendula*, *Polygonum odoratum*, *Brugmansia graveolens*, *Rhus toxicodonta*, *Armoracia rusticana*, *Ficus benjaminii*, *Sufflera* sp, *Baikiaea recurvata*, *Asimina triloba*, *Lippia dulcis*, *Epilobium augustifolium*, *Brugmansia suaveolens*, *Xanthosoma sagittifolium*, *Monstera deliciosa*., *Aglaonema commutatus*, *Dieffenbachia leopoldii*, *Anthurium andreaeanum*, *Syngonium podophyllum*, *Dracaena fragrans*, *Ananas comosus*, *Strelitzia reginae*, *Dieffenbachia segiune*, *Syngonium auritum*, *Dracaena* sp., *Haemanthus katharinae*, *Anthurium altersianum*, *Spathiphyllum grandiflorum*, *Spathiphyllum cochlearispatum*, *Monstera pertusa*, *Anthurium magnificum*, *Anthurium hookeri*, *Anthurium elegans*, *Calathea zebrina*, *Yucca elephantipes*, *Bromelia balansae*, *Musa textilis*, *Myrthus communis*, *Olea*

Oleaster, Olea europaea, Nerium oleander, Cocculus laurifolius, Microsorium punctatum, Sansevieria sp., Adansonia digitata, Boehmeria biloba, Piper nigrum, Phymatosorus scolopendria, Turnera ulmifolia, Nicodemia diversifolia, Tapeinochilos spectabilis, Rauwolfia tetraphylla, Ficus elastica, Cycas circinalis, Caryota urens, Cynnamomum zeylonicum, Aechmea luddemanniana, Phoenix zeylonica, Ficus benjamina, Ficus pumila, Murraya exotica, Trevesia sundaica, Clerodendrum speciosissimum, Actinidia kolomikta, Paeonia lactiflora, Paeonia suffruticosa, Quercus imbricaria, Iris pallida, Portulacca olleracea, Polygonum aviculare, Iris pseudocarpus, Allium nutans, Allium fistulosum, Anthericum ramosum, Veratrum nigrum, Polygonum lapathifolium, Hosta lancifolia, Hosta sieboldii, Echinops sphaerocephalus, Paeonia dahurica, Inula helenium, Crambe pontica, Digitalis lutea, Baptisia australis, Aristolochia australis, Hyssopus seravschanicus, Teucrium chamaedrys, Sedum album, Heracleum pubescens, Origanum vulgare, Cachrys alpina, Laser trilobum, Matteuccia struthiopteris, Sedum telephium, Bocconia cordata, Ajuga reptans, Thalictrum minus, Anemone japonica, Clematis rectae, Alchemilla officinalis, Potentilla alba, Poterium sanguisorba, Menispermum dauricum, Oxybaphus nyctagineus, Armoracia rusticana, Crambe cordifolia, Agrimonia eupatoria, Anchusa officinalis, Polemonium caeruleum, Valeriana officinalis, Pulmonaria mollissima, Stachys lanata, Coronilla varia, Platycarya grandiflora, Lavandula officinalis, Vincetoxicum officinale, Acalypha hispida, Gnetum gnemon, Psychotria nigropunctata, Psychotria metbacteriodomasica, Codiaeum variegatum, Phyllanthus grandifolius, Pterigota alata, Pachyra affinis, Sterculia elata, Philodendron speciosum, Pithecellobium unguis-cati, Sanchezia nobilis, Oreopanax capitatum, Ficus triangularis, Kigelia pinnata, Piper cubeba, Laurus nobilis, Erythrina caffra, Metrosideros excelsa, Osmanthus fragrans, Cupressus sempervirens, Jacobinia sp., Senecio platyphylloides, Livistona chinensis, Tetraclinis articulata, Eucalyptus rudis, Podocarpus spinulosus, Eriobotrya japonica, Ginkgo biloba, Rhododendron sp., Thuja occidentalis, Fagopyrum suffruticosum, Geum macrophyllum, Magnolia kobus, Vinca minor, Convallaria majalis, Corylus avellana, Berberis sp., Rosa multiflora, Ostrya carpinifolia, Ostrya connocea, Quercus rubra, Liriodendron tulipifera, Sorbus aucuparia, Betula nigra, Castanea sativa, Bergeia crassifolia, Artemisia dracunculus, Ruta graveolens, Quercus nigra, Schisandra chinensis, Betula alba, Sambucus nigra, Gentiana cruciata, Encephalartos horridus, Phlebodium aureum, Microlepidium platyphylla, Ceratozamia mexicana, Stenochlaena tenuifolia, Adiantum trapeziforme, Adiantum raddianum, Lygodium japonicum, Pteris

crassifolia, Asplenium australasicum, Agathis robusta, Osmunda regalis, Osmundastrum claytonianum, Phyllitis scolopendrium, Polystichum braunii, Cyrtomium fortunei, Dryopteris filix-mas, Equisetum variegatum, Athyrium nipponicum, Athyrium filix-femina, Parthenocissus tricuspidata, Ligusticum vulgare, Chamaecyparis pisifera, Rosa canina, Cotinus coggygia, Celtis occidentalis, Picea schrenkiana, Cydonia oblonga, Ulmus pumila, Euonymus verrucosus, Deutzia scabra, Mespilus germanica, Quercus castaneifolia, Euonymus europea, Securinega suffruticosa, Koeleria paniculata, Syringa josikaea, Zelkova carpinifolia, Abies cephalonica, Taxus baccata, Taxus cuspidata, Salix babylonica, Thuja occidentalis, Actinidia chinensis, Mahonia aquifolium, Aralia mandshurica, Juglans nigra, Euonymus alata, Prinsepia sinensis, Forsythia europaea, Sorbocotoneaster pozdjakovii, Morus alba, Crataegus macrophyllum, Eucommia ulmifolia, Sorbus commixta, Philodendron amurense, Cornus mas, Kerria japonica, Parrotia persica, Jasminum fruticans, Swida sanguinea, Pentaphylloides fruticosa, Sibiraea altaica, Cerasus japonica, Kolkwitzia amabilis, Amigdalus nana, Acer mandshurica, Salix tamarisifolia, Amelanchier spicata, Cerasus mahaleb, Prunus cerasifera, Corylus avellana, Acer tataricum, Viburnum opulus, Syringa vulgaris, Fraxinus excelsior, Quercus trojana, Chaenomeles superba, Pinus salinifolia, Berberis vulgaris, Cotoneaster horizontalis, Cotoneaster fangianus, Fagus sylvatica, Pinus pumila, Pinus sylvestris and Berberis thunbergii.

An aspect of the invention provides a method for eliciting a compound having therapeutic activity from a plant or plant part, comprising the steps of: contacting a living, intact plant or plant part with an effective amount of acetic acids; and allowing the acetic acid to induce or improve the production of a compound from the plant or plant part. A preferred acetic acid concentration is about 0.1% (v/v) and a preferred aqueous medium is water. A preferred plant portion is a plant root, although leaves and shoots are also contemplated for use in the methods according to the invention. A preferred therapeutic activity is an anti-microbial activity (such as an anti-bacterial activity or an anti-fungal activity) or an anti-cancer activity.



comprises macerating the plant or plant parts in an aqueous medium. Alternatively, the step of extracting the compounds may comprise removing cuticular material located on the surface of a leaf by contacting the leaf surface with a solvent, such as an organic solvent (e.g., methylene chloride or chloroform). Compounds recovered in this manner include, but are not limited to, lipid, wax, cutin, protein, a primary metabolite and/or a secondary metabolite. In some embodiments of the invention, the medium is a liquid medium; in other embodiments, the medium is an agar medium.

In a further related aspect, the above-described method for eliciting a compound having therapeutic activity from a plant or plant part further comprises providing a chemical library of compounds recovered from the aqueous medium in an amount sufficient to assay for biological activity. In some embodiments, the method further comprises assaying the solvent solution for therapeutic activity, such as anti-microbial activity (e.g., anti-bacterial or anti-fungal activity) or anti-cancer activity. In another embodiment, the step of assaying the solvent solution comprises contacting the solution with a medium containing a living microorganism and determining the rate of growth of the microorganism, whereby an inhibition of the growth of the microorganism is indicative of an agent in the solvent solution having therapeutic activity.

Another aspect of the invention provides a method of preparing a composition having therapeutic activity, comprising the steps of: contacting a living intact plant or plant part with an effective amount of acetic acid; allowing the acetic acid to induce a compound or component having therapeutic activity from the plant or plant part; and collecting a composition comprising the compound or component. A preferred concentration of acetic acid for use in the method is about 0.1% (v/v). In one embodiment, the composition is collected by contacting a surface of the plant or plant parts with a solvent suitable for removing cuticular or epicuticular material. In another embodiment, the composition is collected by macerating the plant or plant parts in an aqueous medium.

Numerous additional aspects and advantages of the invention will become apparent to those skilled in the art upon consideration of the following detailed description of the invention.

### BRIEF DESCRIPTION OF THE DRAWING

Figure 1 is a graphical presentation of the amount of daidzein recovered from root exudates produced by soybean plants treated with different elicitors in accordance with an embodiment of the invention.

Figure 2 is a graphical presentation of the amount of genistein recovered from root exudates produced by soybean plants treated with different elicitors in accordance with an embodiment of the invention.

Figure 3 is an HPLC profile of the diversity of compounds recovered from root exudates of various plants in accordance with an embodiment of the invention.

Figure 4 is an HPLC profile of the diversity of compounds recovered from *Lupinus luteus* in accordance with an embodiment of the invention.

Figure 5 is an HPLC profile of the diversity of compounds recovered from root exudates of *Brassica juncea* in accordance with an embodiment of the invention.

Figure 6 is an HPLC profile of the diversity of compounds recovered from root exudates of *Datura metel* in accordance with an embodiment of the invention.

Figure 7 is an HPLC profile of the diversity of compounds recovered from root exudates of *Lupinus polyphyllus* in accordance with an embodiment of the invention.

Figure 8 is an HPLC profile of the diversity of compounds recovered from root exudates of *Melilotus medicaginoides* in accordance with an embodiment of the invention.

Figure 9 is an HPLC profile of the diversity of compounds recovered from *Solanum melongena* without treating with an elicitor.

Figure 10 is an HPLC profile of the diversity of compounds recovered from root extracts from *Solanum melongena* which was treated with an elicitor.

Figure 11 is an HPLC profile of the diversity of compounds recovered from *Daucus carota* which was treated with an elicitor.

Figure 12 is an HPLC profile of the diversity of compounds recovered from *Glycyne max* which was treated with elicitors, as compared to a control.

Figure 13 is an HPLC profile of the diversity of compounds recovered from *Daucus carota* which was treated with elicitors, as compared to a control.

Figure 14 is an HPLC profile of the diversity of compounds recovered from *Daucus carota* which was treated with elicitors, as compared to a control.

Figure 15 is an HPLC profile of the diversity of compounds recovered from *Lycopersicon esculentum* which was treated with elicitors, as compared to a control.

Figure 16 is an HPLC profile of the diversity of compounds recovered from *Lupinus polyphyllus* which was treated with elicitors, as compared to a control.

Figure 17 is a representation of an agar plate showing antimicrobial activity of the root exudate of *Laurus nobilis* (1881) against *Escherichia coli*.

Figure 18 is a representation of an agar plate showing antimicrobial activity of the root exudate of *Gentiana tibetica* (1881) against *Escherichia coli*.

Figure 19 is a representation of an agar plate showing antimicrobial activity of the root exudate of *Aconitum napellus* (1881) against *Escherichia coli*.

Figure 20 is a representation of an agar plate showing antimicrobial activity of the leaf surface compounds (identified on the Figure as samples) of *Erythrina christa-galli* (1363) against *Staphylococcus aureus*.

Figure 21 is a representation of an agar plate showing antimicrobial activity of the leaf surface compounds (identified on the Figure as samples) of *Laurus nobilis* (1513) against *Staphylococcus aureus*.

Figure 22 is a representation of an agar plate showing antimicrobial activity of the root exudate of *Scutellaria altissima* (1671) against *Staphylococcus aureus*.

Figure 23 is a representation of an agar plate showing antimicrobial activity of the root exudate of *Scutellaria cretica* (1691) against *Staphylococcus aureus*.

Figure 24 is a representation of an agar plate showing antimicrobial activity of the root exudate of *Hosta fortunei* (1645) against *Saccharomyces cerevisiae*.

Figure 25 is a representation of an agar plate showing antimicrobial activity of the root exudate of *Cunninghamia lanceolata* (2489) against *Aspergillus flavus*.

Figure 26 is a representation of an agar plate showing antimicrobial activity of leaf surface compounds (identified on the Figure as samples) from leaves of *Thymus citriodorus* "aureus" (59) and *Hydrocotyle asiatica* (32a) against *Staphylococcus aureus*.

Figure 27 is a representation of an agar plate showing antimicrobial activity of leaf surface compounds (identified on the Figure as samples) from leaves of *Betula pendula* (24) against *Staphylococcus aureus*.

Figure 28 is a representation of an agar plate showing antimicrobial activity of leaf surface compounds of *Eucalyptus rudis* (229) against *Staphylococcus aureus*.

Figure 29 is a representation of an agar plate showing antimicrobial activity of leaf surface compounds of *Eucalyptus rudis* (229) against *Saccharomyces cerevisiae*.

Figure 30 is a representation of an agar plate showing antimicrobial activity of leaf surface compounds of *Oreopanax capitatus* (216) against *Staphylococcus aureus*.

Figure 31 is a representation of an agar plate showing antimicrobial activity of leaf surface compounds of *Oreopanax capitatus* (216) against *Escherichia coli*.

### DETAILED DESCRIPTION OF THE INVENTION

The plant used in the present invention may be an entire grown plant or a plant seed or seedling or a plant shoot or root. The plant or plant portion is maintained intact and alive and is capable of being sustained without either an organic nutritional supplement or without maintaining sterile conditions for the plant. If desired or preferred, inorganic supplements may be employed in order to increase plant growth, but are not necessary to the method of the invention.

Typically, the plant or plant part is grown and maintained in a growth state similar to that in a natural surrounding. The plant functions as a natural bioreactor for producing valuable plant products, which may be recovered by contacting the plant or plant part with an aqueous medium, for example water. Moreover, the plants are capable of being grown and maintained in a manner of continuous growth, i.e., without destroying the plant. The plant or plant parts can be grown without any organic supplements although an organic supplement could be used if desired. As such the plant or plant parts are different than plants or plant parts that are cultured in a laboratory which generally require organic nutritional supplements and sterile conditions in order to maintain growth.

As herein above indicated, the plants or plant parts may be subjected to physical or chemical treatment to elicit or induce an increased production of one or more compounds. The plants, and particularly in the roots of the plant may be contacted with any elicitor or inducer. Physical elicitors can include treatment such as ultra-violet radiation; low and high temperature stress; osmotic stress, for example as induced by salt or sugars; and nutritional stress, such as depriving the plant of essential nutrients (N, P, or K). More specifically, compounds that have demonstrated effectiveness in inducing the production of compounds with therapeutic activity in plants include, but are not limited to, salicylic acid, acetic acid, silver nitrate, chitosan, N-hexanoyl homericinellactone, methyl jasmonate, tetcyclases,

pentafluorobenzoic acid, dihydroxybenzoic acid, cinnamic acid, 2-fluorobenzoic acid, sodium fluoride, yeast extract, laminarin, SDS, jasmonic acid, okadaic acid, polygalacturonic acid, 1-phosphatidic acid, polyethylene glycol, hydrogen peroxide, paraquat, calyculin A, 1-aminobutyrate, eicosapentanoic acid, arachidonic acid, glutathione, ascorbic acid, nickel, copper, and lead. Chemical compounds that can elicit or induce compounds with therapeutic activity includes, but are not limited to organic and inorganic acids, fatty acids, glycerides, phospholipids, glycolipids, organic solvents, amino acids peptides, monosaccharides, oligosaccharides, polysaccharides, lipopolysaccharides, phenolics, alkaloids, terpenes, terpenoids, antibiotics, detergents, polyamines, peroxides, ionophores, and the like.

For example, a glycopeptide elicitor may be prepared from germ tubes of the rust fungus *Puccinia graminis* Pers. F. sp. *Tritici* Erkss. & Henn (Pgt), as well as chitin oligosaccharides, chitosan, and methyl jasmonate (MG) stimulated lipoxygenase (LOX) activity (E.C. 1.13.11.12) in wheat (*Triticum aestivum*) leaves. The effects of elicitor concentration and exposure time on growth and levels of biologically active compounds vary. For example, transient studies at the same level demonstrated possible catabolism as serpentine, tabersonine, and lochnericine levels decreased immediately after elicitation. The levels of these compounds recovered back to control levels or were higher than control levels after some time. Jasmonic acid was found to be a unique elicitor leading to an enhancement in flux to several branches in the indole alkaloid pathway. Jasmonic acid addition caused an increase in the specific yields of ajmalicine (80%), serpentine (60%), lochnericine (150%), and horhammericine (500%) in dosage studies. Tabersonine, the likely precursor of lochnericine and horhammericine, decreased at lower levels of jasmonic acid and then increased with increasing jasmonic acid concentration. Transient studies showed that lochnericine and tabersonine levels go through a maxima, then decrease back to control levels and reduce below control levels, respectively. The yields of ajmalicine, serpentine, and horhammericine increased continuously after the addition of jasmonic acid. The methods described in the invention could generally be used in devising strategies for enhancement in productivity of secondary metabolites and for probing and studying the complex secondary metabolite pathways in plant tissue cultures.

The elicitor treatment may be applied continuously or intermittently to the plant or plant part. In one embodiment, such treatment may be accomplished by contacting the plant roots with a solution containing the elicitor or by irradiating the roots or exposing them to

other environmental stresses, for example temperature stresses; however, the invention is not limited to such an embodiment in that other portions of a plant or seedlings may be contacted with an elicitor.

One elicitor, acetic acid, demonstrates particular effectiveness for inducing the production of compounds having therapeutic activity in plants. The exposure of plants or plant parts to acetic acid induced compounds demonstrating cytotoxicity against breast, central nervous system, and lung cancer cells in at least 40% of plant species tested. In addition, at least 30% of plant species exposed to an acetic acid elicitor demonstrated antimicrobial activity, for example activity against gram-positive bacteria, gram-negative bacteria, or inhibition of yeast or fungal growth. While not intended to be limited by any theory of the invention, it is particularly interesting to note that the effects of elicitation by acetic acid in plants or plant parts is not limited to, or necessarily, correlated with any change in the pH of the plant environment, as would be expected by one of skill in the art. Moreover, although acetic acid may be a very basic precursor of the indole-3-acetic acid pathway in plants, to the best knowledge of the applicants the literature has neither suggested nor reported any utility of acetic acid as a precursor.

The elicitor is applied in any amount suitable for inducing or improving production of a compound having therapeutic activity in the plant or plant parts. As used herein, the term “effective amount” refers to any amount capable of inducing or improving particularly antibacterial, anti-fungal, or anticancer activity in the subject plant or plant parts.

The present invention may be employed for screening for potentially valuable products. The plant or plant part subjected to the elicitor treatments can be extracted into water or recovered from harvested roots as herein above described.

Generally, the living plant or plant part is contacted with water or other aqueous extraction medium. The living water or extraction medium containing the secreted phytochemicals is then analyzed to ascertain whether or not the medium contains a potentially valuable chemical compound, i.e. a compound demonstrating therapeutic activity. The contact with water may be effected by placing at least the plant roots in water or by "aeroponics," which involves contacting the plant, in particular, the roots of the plant, with water droplets from which chemical compounds is recovered. The living plant or plant part subjected to the process of the invention is an intact plant or plant part in the form of an entire plant or plant seedlings including for example seeds, leaves, or plant roots. In each case, the plant or plant

part is contacted with water, which may be pure water or water containing appropriate additives, such as desired chemical elicitors. In the case where a whole plant is used, it is preferred that the plant roots are contacted with water in order to recover chemical products.

In accordance with a preferred aspect, the chemical products are those which are secreted or leached from the plant, either the seedling or a shoot, and in particular, the plant root. In the case where seedlings, seeds, or shoots are used, and the plant, seed, shoot or root may be treated with an elicitor or inducer to increase production in the plant of one or more products. The inducer or elicitor may be present in the water that contacts the plant for chemical recovery or may be separately applied to the plant in any suitable manner.

In accordance with one preferred aspect of the invention, secreted chemical products are recovered from a plant (or plant portions) in a continuous process contacting the plant or plant parts with an aqueous or in a medium continuous or periodical manner to dissolve the chemicals secreted by the plant or plant parts into the aqueous medium. The aqueous medium containing exuded or secreted chemical products is then treated to recover the products from the water.

Alternatively, the roots are harvested and the chemicals are recovered from the harvested root by extracting and macerating the root tissue in an aqueous medium. In such embodiment, the plant or rooted shoots may be grown hydroponically and the plants are cultivated on top of a mesh with a small portion of their root system anchored in a layer of artificial soil above the mesh. The artificial soil layer is employed to supply all essential nutrients to the plants. In such an embodiment, a major portion of the root system grows through the mesh and soil layer into water, or other aqueous medium, which is below the mesh layer. The water flows over the roots in order to recover exuded chemicals. The water containing the exuded chemicals is then treated to recover and/or isolate the chemicals therefrom. In another embodiment, the root is harvested and chemicals are recovered from the harvested roots.

In a further embodiment, plants may be germinated and supported in rockwool cubes, as known in the art, with roots extending into a water solution which typically contains the elicitor and any inorganic nutrients.

In another embodiment, plant seedlings are employed as a source of the phytochemicals. In such an embodiment, seeds are germinated in aerated water, with the water being recovered on a continuous or semi-continuous basis in order to treat the water to

obtain therefrom secreted phytochemicals. Although the preferred embodiment is a continuous process, batch processes may also be employed for accomplishing the method of the invention.

Thus, in accordance with the present invention, plants or plant parts may be grown on a large scale and used to effectively generate a diverse library of compounds for screening for various applications. Such diversity may be obtained by using a variety of plants and/or a variety of elicitors. Such library may be effectively generated and screened in that such variety of compounds may be recovered as root exudates by a simple water extraction. Moreover, the extraction medium may also contain the elicitors which function to elicit various compounds in the root exudates.

Furthermore, the present invention provides a "factory" for large scale production of compounds in that desired compounds can be simply recovered in root exudates from plants or plant parts which are hydroponically grown. Moreover, by use of a selected elicitor, desired compounds which may not be normally present in root exudates and/or which may not be present in sufficient quantities in root exudates can be recovered on a large scale from root exudates produced by hydroponically grown plants or plant parts by a simple water extraction or by harvesting roots without destroying the plant.

The water or aqueous medium which contains the secreted chemicals also can be treated to recover the chemicals therefrom. The chemical compounds are recovered from the water or extraction medium used on harvested roots. For example, in one aspect, the invention provides a method of identifying an agent which is exuded onto the surface of a leaf of a plant having therapeutic activity. The method comprises (a) removing cuticular material located on the surface of the leaf, comprising contacting the leaf surface with a solvent, thereby resulting in a solvent solution; (b) assaying the solvent solution for the identification of an agent which has therapeutic activity; and (c) analyzing the solvent solution so as to identify the agent which has the therapeutic activity. The extraction medium is analyzed by various techniques in order to assist in identifying the recovered compounds. For example, the chemicals may be recovered by column chromatography, crystallization, distillation, liquid or solid phase extraction and the like. Such procedures are known in the art and should be apparent to those skilled in the art from the teachings herein.



The cuticular material can be a lipid, wax, cutin, protein, primary or secondary metabolite. Typically, the solvent is an organic solvent. Examples of solvents include, but are not limited to, methylene chloride and chloroform.

The step of analyzing the solution further can include fractioning the solvent solution directly or following any drying or resuspension step. In one embodiment, the resulting agent is identified subsequent to fractioning the solution. Fractionation methods are known to those skilled in the art. For example, chromatographic methods, for example HPLC, may be employed to identify the compound. In particular, the chromatography separation of extracted products may be employed with an HPLC-system consisting of Waters 996 Photodiode Array Detector (PDA) with usable UV range from 190 to 800 nm; a Waters 717 plus autosampler; two Beckman and a Beckman System Gold Analog Interface 406. The Beckman solvent delivery system is controlled by a NEC PC-8300 computer. Chromatography and spectral data is managed by Waters Millennium chromatography software, version 2.10, using a NEC Image 466es computer. All hardware components, except the solvent delivery system, are connected through a standard IEEE 488 communication system. Compounds are separated on a Waters Nova Pak® C-18 reverse phase column, 3.9 x 150 mm, 60 Å pore size, and 4 µm particle size. Prior to use, each batch of solvent A is digested under vacuum and ultrasonication for 5 minutes. The mobile phase flow is adjusted to 1 mL/min, and a gradient mode of separation is used for all separations. Compounds are detected with PDA detector or with Waters™ Thermabeam Mass Detector.

Further, one may fractionate the sample by chromatography techniques, followed by the chemical structure analysis using mass spectroscopy; infrared spectroscopy; or 1D or 2D nuclear magnetic resonance spectroscopy (proton or <sup>13</sup>C). Fractionation and analysis methods also are known to those skilled in the art.

As used herein, the term “therapeutic activity” refers to a biological activity, typically selected from antibacterial activity, antifungal activity, or anticancer activity. Particularly, “anti-microbial activity” as used herein can identify anti-bacterial and anti-fungal activity. Therapeutic activity can be identified by contacting the solvent solution, aqueous medium, or its components with a media containing a suspension of a microorganism, wherein the inhibition of the growth of the suspension of the microorganism is indicative of an agent in the solvent solution having therapeutic activity. The medium may be a liquid media or an agar media. Microorganisms can include, but not limited to any bacteria or fungi that grow

within or develop in or on the media. Inhibition of the growth is detected by standard means known to those skilled in the art. For example, the growth inhibition on agar may be measured in terms of zone of inhibition, which is known to those skilled in the art.

The microorganism can be selected from a gram positive or a gram negative bacteria, protozoan, fungus, or virus. As defined herein, "antimicrobials" means the spectrum of organisms against which they are active, whether they kill the organism or merely slow its growth and reproduction (i.e., cidal or static), and the biochemical system on which they exert their major biochemical action (e.g., inhibit protein synthesis or cell wall synthesis). Antimicrobials can include gram positive or gram negative bacteria and may even act against such organisms as Rickettsia, fungi, or protozoans. Examples of gram positive and gram negative bacteria are known to those skilled in the art. Microorganisms include, but are not limited to, *Escherichia coli* K-12 F, prototropic Str, *Staphylococcus aureus* subsp. *Aureus*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*, *Aspergillus flavus*, and *Penicillium Nigra*.

Two common applications of agar diffusion assays are potency testing of new production lots in the pharmaceutical industry and bacterial susceptibility testing. Such applications are based on the same principles, but susceptibility assays are unknown bacterial strains, and potency assays use bacterial strains with well-characterized performance against the test drug. Agar diffusion potency assays are relatively comparable in sensitivity and accuracy to radiometric (RIA) enzyme, fluorescent (FIA) enzyme, & ELISA. For example, single-plate assay is based on having all standard and unknown concentrations on one single plate. This eliminates plate to plate variation, and facilitates layout and reading. Alternatively, 2 or 3 identical plates may be laid out, read and averaged. Usually NUNC (Denmark) large 24.3 mm square plates are used, that allow up to 64 samples that easily fit on one plate. As such, the assay provides for up to 6 standards, and up to 10 samples, with 4 replicates of each standard and unknown sample concentrations, on one plate. Multiple-Plate format uses many 90-100 mm petri dishes, and conforms strictly to US-FDA, US-CFR, and US-USP published methodology. Zone diameters are measured by Video or Caliper directly into the software.

In one embodiment the plant is a higher plant. The use of any plant is contemplated and may be employed in the method of the invention. For example, the following plants may be employed in order to identify agents which are produced by elicitation: *Atropa belladonna*,

*Erythrina flabelliformis*, *Ipomoea tricolor*, *Erythrina crista*, *Celosia cristata*, *Gallium spurium*, *Laurus nobilis*, *Vitis labrusca*, *Gratiola officinalis*, *Symphitum officinalis*, *Hosta fortunei*, *Cassia hebecarpa*, *Thalictrum flavum*, *Scutellaria altissima*, *Portulacca oleracea*, *Scutellaria certicola*, *Physalis cretica*, *Geum fauriei*, *Gentiana tibetica*, *Linum hirsutum*, *Aconitum napellus*, *Podophyllum emodii*, *Thymus cretaceus*, *Hosta fortunei*, *Carlina acaulis*, *Chamaecrista fasciculata*, *Pinus pinea*, *Peganum harmala*, *Tamarindus indica*, *Carica papaya*, *Cistus incanus*, *Capparis inermis*, *Cupressus lusitanica*, *Diospyros kaki*, *Eryngium campestre*, *Aesculus woerlitzensis*, *Aesculus hippocastanum*, *Cupressus sempervirens*, *Celtis occidentalis*.

Further plants species used for screening are as follows: *Polygonum cuspidatum*, *Elaeagnus angustifolia*, *Elaeagnus commutata*, *Gentiana macrophylla*, *Brassica rapa*, *Sesbania exaltata*, *Sesbania speciosa*, *Spartina potentiflora*, *Brassica juncea*, *Helianthus annuus*, *Poinsettia* sp., *Pelargonium zonale*, *Leontopodium alpinum*, *Lupinus luteus*, *Buxus microphylla*, *Liatris spicata*, *Primula japonica*, *Betula nigra*, *Filipendula vulgaris*, *Lobelia siphilitica*, *Grevillea robusta*, *Reseda luteola*, *Gentiana littoralis*, *Campanula carpatica*, *Aesculus hippocastanum*, *Aesculus woerlitzensis*, *Ageratum conyzoides*, *Psidium guajava*, *Ailanthus altissima*, *Buxus microphylla*, *Hydrocotyle asiatica*, *Grevillea robusta*, *Brugmansia suaveolens*, *Thymus pulegioides*, *Thymus lema-barona*, *Thymus serpyllum*, *Gaultheria procumbens*, *Thymus carnosus*, *Thymus thracicus*, *Calycanthus floridus*, *Zingiber officinalis*, *Lamium dulcis*, *Thymus praecox*, *Thymus pulegioides*, *Thymus speciosa*, *Thymus carnosus*, *Thymus pseudolamginosus*, *Thymus vulgaris*, *Ficus religiosa*, *Forsythia suspensa*, *Chelidonium majus*, *Thymus wooly*, *Thymus portugalense*, *Nicotiana tabacum*, *Thymus cytidorus*, *Cactus officinailis*, *Lablab purpurea*, *Juglans regia*, *Actinidia chinensis*, *Hemerocallis*, *Betula pendula*, *Gardenia jasminoides*, *Taxodium distichum*, *Magnolia loebherii*, *Crataegus praegophyrum*, *Larix decidua*, *Thuja orientalis*, *Thuja occidentalis*, *Thuja orientalis*, *Cupressocyparis leylandii*, *Pseudotsuga menziesii*, *Abies firma*, *Parthenocissus quinquefolia*, *Allium cernuum*, *Juniperus "blue pacific"*, *Taraxacum officinalis*, *Yucca* sp., *Ilex aquifolium*, *Tsuga canadensis*, *Ilex cornuta*, *Taxus hicksii*, *Taxus media*, *Metasequoia glyptostroboides*, *Pinus bungiana*, *Buxus sempervirens*, *Stewartia koreana*, *Prunus* sp., *Betula dahurica*, *Plantago minor*, *Acer palmatum*, *Acer campestre*, *Cotynus coggygria*, *Quercus robur*, *Acer truncatum*, *Achyranthes bidentata*, *Allium japonicum*, *Carum capsicum*, *Agastache mexicana*, *Prunella vulgaris*, *Tagetes minuta*, *Nepeta*

cataria, Ratibida columnifera, Aster novae angliae, Myrica cerifera, Pittosporum tobira, Taxodium distichum, Plantago major, Pinus sylvestris, Acorus canadensis, Pieris japonica, Pinus strobus, Trifolium pratense, Prunus serotica, Datura stramonium, Geranium maculata, Hydrocotyle asiatica, Taxodium distichum, Astragalus sinicus, Centaurea maculata, Ruschia indurata, Myrthus communis, Platanus occidentalis, Lycium barbarum, Lavandula officinalis, Grevillea robusta, Hippophae rhamnoides, Lipidendula ulmaria, Betula pendula, Polygonum odoratum, Brugmansia graveolens, Rhus toxicodonta, Armoracia rusticana, Ficus benjamina, Sluffera sp, Pelagonium zonale, Allium sp, Asimina triloba, Lippia dulcis, Epilobium angustifolium, Brugmansia suaveolens (old), Brugmansia suaveolens (young), Xanthosoma sagittifolium. (leaf), Xanthosoma sagittifolium (stem), Monstera deliciosa., Aglaonema commutatus, Dieffenbachia leopoldii, Anthurium andreanum, Syngonium podophyllum, Dracaena fragrans, Ananas comosus, Strelitzia reginae, Dieffenbachia seguine, Syngonium auratum, Dracaena sp, haemanthus kaffir, Anthurium andraeanum, Spathiphyllum grandiflorum, Spathiphyllum. cochlearispatum, Monstera, pertusa, Anthurium magnificum, Anthurium hookeri, Anthurium elegans, Calathea zebrina, Yucca elephantipes, Bromelia balansae, Musa textilis (Leaf), Musa textilis (Stem), Myrthus communis, Olea oleaster, Olea europaea, Nerium oleander, Cocculus laurifolius, Microsorium punctatum, Ficus sp., Sensevieria sp., Adansonia digitata, Boechimeria boloba, Piper nigrum, Phymatosorus scolopendria, Turnera ulmifolia, Nicotiana glauca, Tapeinochilos spectabilis, Rauwolfia tetraphylla, Ficus elastica, Cycas revoluta, Caryota urens, Cinnamomum zeylonicum, Aechmea lueddemanniana, Phoenix dactylifera, Ficus benjamina, Ficus pumila, Murraya exoniensis, Trevesia laurifolia, Clerodendrum speciosissimum, Actinidia chinensis, Paeonia officinalis, Paeonia moutan, Paeonia officinalis, Quercus imbricaria, Iris alba, Portulacca oleracea, Polygonum aviculare, Iris pseudocarpus, Allium nutans, Allium fistulosum, Antirrhinum majus, Veratrum nigrum, Polygonum latifolia, Hosta lanceifolia, Hosta zibaldii, Echinops sp., Echinops dahuricus, Inula helenium, Thymus praecox, Digitalis purpurea, Bactris australis, Austrocyathus australis, Hesperis matronalis, Feutria ham. edris., Sedum album, Heracleum pubescens, Origanum vulgare, Cactis alpina, Hesperis trilobum, Matteuccia struthiopteris, Sedum telchium, Bocconia cordata, Ajuga reptans, Thalictrum minus, Anemone japonica, Clematis recta, Thalictrum sp., Alchemilla sp., Potentilla alba, Poterium sanguisorba, Nispermum dauricum, Oxybaphus nocturnus. Armoracia rusticana, Crambe cordifolia. A. rimonia eupatoria, Anchusa officinalis, Poly monium ceruleum,

*Valeriana officinalis*, *Pulmonaria molissima*, *Stachys lanata*, *Coronilla varia*, *Platycarya grandiflora*, *Lavandula officinalis*, *Vincetoxicum officinale*, *Acalypha hispida*, *Gnetum gnemon*, *Psychotria nigropunctata*, *Psychotria metbacteriodomasica*, *Codiaeum variegatum*, *Phyllanthus grandifolius*, *Pterigota alata*, *Pachyra affinis*, *Sterculia elata*, *Philodendron speciosum*, *Pithecellobium unguis-cati*, *Sanchezia nobilis*, *Oreopanax capitatus*, *Ficus triangularis*, *Kigelia pinnata*, *Piper cubeba*, *Laurus nobilis*, *Erythrina caffra*, *Metrosideros excelsa*, *Osmanthus fragrans*, *Cupressus sempervirens*, *Jacobinia* sp., *Senecio platyphylloides*, *Livistona chinensis*, *Tetraclinis articulata*, *Eucalyptus rudis*, *Podocarpus spinulosus*, *Eriobotrya japonica*, *Ginkgo biloba*, *Rhododendron*, *Thuja occidentalis*, *Fagopyrum suffruticosum*, *Geum macrophyllum*, *Magnolia kobus*, *Vinca minor*, *Convallaria majalis*, *Corylus avellana*, *Berberis* sp., *Rosa multiflora*, *Ostrya carpinifolia*, *Ostrya connogea*, *Quercus rubra*, *Liriodendron tulipifera*, *Sorbus aucuparia*, *Betula nigra* (leaf), *Betula nigra* (flower), *Castanea sativa*, *Bergenia crassifolia*, *Artemisia dracunculus*, *Ruta graveolens*, *Quercus nigra*, *Schisandra chinensis*, *Betula alba*, *Sambucus nigra*, *Gentiana cruciata*, *Encephalartos horridus*, *Phlebodium aureum*, *Microlepia platyphylla*, *Ceratozamia mexicana*, *Stenochlaena tenuifolia*, *Adiantum trapeziforme*, *Adiantum raddianum*, *Lygodium japonicum*, *Pessopteris crassifolia*, *Asplenium australasicum*, *Agathis robusta*, *Osmunda regalis*, *Osmundastrum claytonianum*, *Phyllitis scolopendrium*, *Polystichum braunii*, *Cyrtomium fortunei*, *Dryopteris filix-mas*, *Equisetum variegatum*, *Athyrium nipponicum*, *Athyrium filix-femina*, *Parthenocissus tricuspidata*, *Ligusticum vulgare*, *Chamaecyparis pisifera*, *Rosa canina*, *Cotinus coggygria*, *Pinus strobus*, *Celtis occidentalis*, *Picea schrenkiana*, *Cydonia oblonga*, *Ulmus pumila*, *Euonymus verrucosus*, *Deutzia scabra*, *Mespilus germanica*, *Quercus castaneifolia*, *Euonymus europea*, *Securinega suffruticosa*, *Koeleruteria paniculata*, *Syringa josikaea*, *Zelkova carpinifolia*, *Abies cephalonica*, *Taxus baccata*, *Taxus cuspidata*, *Salix babylonica*, *Thuja occidentalis*, *Actinidia kolomicta*, *Mahonia aquifolium*, *Aralia mandschurica*, *Juglans nigra*, *Euonymus elata*, *Prinsepia sinensis*, *Forsythia europaea*, *Sorbotoconeaster pozdnjakovii*, *Morus alba*, *Crataegus macrophyllum*, *Eucommia ulmifolia*, *Sorbus commixta*, *Philodendron amurense*, *Cornus mas*, *Kerria japonica*, *Parrotia persica*, *Jasminum fruticans*, *Swida sanguinea*, *Pentaphylloides fruticosa*, *Sibiraea altaiensis*, *Cerasus japonica*, *Kolkwitzia amabilis*, *Amigdalus nana*, *Acer mandschurica*, *Salix tamarisifolia*, *Amelanchier spicata*, *Cerasus mahaleb*, *Prunus cerasifera*, *Corylus avellana*, *Acer tataricum*, *Viburnum opulus*, *Syringa vulgaris*, *Fraxinus exelsior*,

*Quercus trojana*, *Chaenomeles superba*, *Pinus salinifolia*, *Berberis vulgaris*, *Cotoneaster horizontalis*, *Cotoneaster fangianus*, *Fagus sylvatica*, *Pinus pumila*, *Pinus sylvestris*, and *Berberis thunbergii*.

The invention will be further described with respect to the following examples. The examples are intended to provide an illustration of the invention and should not be construed as a limitation of the invention in any way.

## EXAMPLES

### **Example 1. Plant Production**

Seeds were germinated in a greenhouse equipped with supplementary lighting (16-h photoperiod 24-28°C). Seeds were placed inside 0.9 cm diameter, 0.9 cm deep well drilled in Grodan rockwool cubes (3.4 cm width x 3.4 cm depth x 3.7 cm height) purchased from Grodania A/S, Hedehusene, Denmark.

Depending on the speed of germination, the seeds were either placed directly into the rockwool cubes or sterilized to prevent rotting during the germination process. For sterilization, seeds were immersed first in 70% ethyl-Alcohol for 10-15 seconds, then in 2.5% Sodium Hypochlorite for 10-15 min., and finally rinsed thoroughly with distilled water. The sterilized seeds were placed in a Petri dish lined with no. 1 Wattman paper (Wattman International Ltd., Maidstone, England), soaked in either a sterile water for seeds larger than 1 mm in diameter, or for smaller seeds with mineral salts nutrient solution. The Petri dishes were sealed with parafilm before being placed in a growth chamber (12-h photoperiod 22-24°C) until the seeds germinated.

Rockwool cubes were placed inside standard greenhouse plastic trays (dimensions 52 cm width x 25 cm depth x 7 cm height) and watered with an intermittently operating overhead misting system triggered by a moisture sensor (Mist-A-Matic, E.C. Geiger Inc., Larleysville, PA). Seeds were allowed to germinate for 3-6 days till the roots started to emerge from the bottom of the rockwool cube.

After germination, the cubes with the seedlings were inserted into a 3.2 cm diameter round opening cut in the center of Styrofoam ring (8.2 cm diameter, 2.5 cm thickness). The ring was floated on the surface of 400-800 mL of hydroponic nutrient solution (2 g/L Hydro-Sol [Scotts-Sierra Horticultural Products Comp., Marysville, OH] supplemented with 1.2 g/L

Ca[NO<sub>3</sub>]<sub>2</sub>) containing inside light impermeable, high-density polyethylene cylinder (9.0 cm in diameter, 16 cm in height).

Aeration was provided either by shaking the cylinders at 50 rpm on the platform shaker (Model Orbit, Lab-Line Instruments, Inc., Melrose Park, IL) or by bubbling compressed air through the solution. Seedlings were cultivated hydroponically in this system for 3 to 5 weeks with roots growing in a nutrient solution. Thereafter, the root system (average root dry weight  $0.1 \pm 0.05$  g) was removed from the nutrient solution and placed inside a 30 mL glass beaker, containing 10-20 mL of distilled water or distilled water supplemented with the elicitor. To prevent the water loss from the plant canopy and the drying of the collecting solution, shoots of the plants were covered with a plastic bag. After 24 h, unless noted otherwise, a small sample from the root solution was removed and analyzed for the phytosecreted products.

#### **Example 2. Flow-through phytosecretion system**

The flow-through phytosecretion system consisted of a stainless steel container (53 cm width x 34 cm depth x 20 cm height) with 15-24 soybean plants (glycine max) supported by the rockwool cubes inserted in the openings in the Styrofoam raft (5.0 cm thickness) which had dimensions slightly smaller than the internal dimensions of the container. This Styrofoam raft was floating on top of approximately 10 L of nutrient solution (2 g/L Hydro-Sol supplemented with 1.2 g/L Ca(NO<sub>3</sub>)<sub>2</sub>, aerated with compressed air supplied through an air hose placed on the bottom of the container. After 4-5 weeks, or when the roots reached the appropriate size **[PLEASE DEFINE-- SIZE EQUIVALENT TO \_\_\_\_ ROOTS GROWN AS DESCRIBED FOR 4-5 WEEKS?]**, the volume of nutrient solution was reduced to 2 L. The flow of the nutrient solution, with or without an elicitor, through the flow through system was maintained with a peristaltic pump (Variable Flow Mini-Pump, Fisher Scientific, Pittsburgh, PA), which allowed easy adjustments in the volume of the solution entering the system. Typically, flow rates used in the experiments ranged from 1.5 to 4.5 L/day. The intake tube of the peristaltic pump was immersed in a 60 L plastic storage container containing nutrient solution. Solution from the storage container dripped into the phytosecretion system through the tube attached to its wall. When necessary, elicitors were added to the storage container at the desired concentration. The solution was discharged from the phytosecretion container in the side opposite to the inlet through the opening cut in

the bottom of the container. Solution level in the phytosecretion container was adjusted by changing the height of the opening of the outlet tube. Solution samples were taken from the end of the outlet tube at the specific intervals and analyzed for the presence of the phytosecreted compounds.

**Example 3. High-pressure liquid chromatography (HPLC) analysis of phytosecreted natural products (isoflavonoids)**

Soybean (glycine Max) seeds were germinated and a root solution was removed as described in Example 1.

An HPLC method for separation and identification of phytosecreted compounds, using isoflavonoids daidzein and genistein is used as an example. The chromatography separation was performed with an HPLC-system consisting of Waters 996 Photodiode Array Detector (PDA) with usable UV range from 190 to 800 nm; a Waters 717 plus autosampler; two Beckman 110B solvent Delivery Modules, connected with a Beckman System Organizer (mixer) and a Beckman System Gold Analog Interface Module 406. The Beckman solvent delivery system was controlled by a NEC PC-8300 computer. Chromatography and spectral data was managed by Waters Millennium chromatography software, version 2.10, using a NEC image 466es computer. All hardware components, except the solvent delivery system, were connected through a standard IEEE communication system. Isoflavonoid compounds were separated on a Waters Nova Pak® C-18 reverse phase column, 3.9 x 150 mm, 60 Å pore size, and 4 µm particle size.

The mobile phase consisted of two components: Solvent A - 0.5% ACS grade acetic acid in double distilled water, pH 3-3.5; and Solvent B - acetonitrile. Prior to use, each batch of solvent A was degassed under vacuum and ultrasonication for 5 minutes.

The mobile phase flow was adjusted to 1 mL/min, and a gradient mode of separation was used for all separations. The gradient profile was as follows:

- 0 - 20 min 0% B - 100% B;
- 20 - 22 min 100% B;
- 22 -25 min 100% B - 0% B;
- 25 - 33 min 0% B (column equilibration for next injection).

Compounds were detected with PDA detector within the wavelength range of 200 to 400 nm. The column temperature was ambient.



Under the above conditions, daidzein had retention time 11.725 min and UV maxima at 250.9 nm and 302.9 nm and genistein had a RT of 12.94 min and UV maximum at 260.3 nm. Depending on the resolution setting of the PDA detector, a negligible shift of  $\pm 3$  nm in the absorbance maxima was observed. A  $\pm 0.5$  min of tolerance in the retention times with the different batches of solvents was detected.

All plants were grown hydroponically, as previously described, and phytosecreted compounds collected for 24 hours in distilled water containing an elicitor or mixtures of different elicitors, except for treatment no. 47, where no elicitors were present in the collecting water. Daidzein and genistein content in root exudates from un-elicited plants grown under the same conditions was below the detection limits - 400 pg, or 4  $\mu\text{g/L}$  for daidzein, and 25 pg, or 250 ng/L for genistein.

#### Elicitor treatments:

- 3 - Salicylic acid (5 mM), Tetcyclases (0.2 mM) and 7.5% ethanol (EtOH)
- 5 - Salicylic acid (5 mM) and 5% EtOH
- 6 - Salicylic acid (2.5 mM) and 2.5% EtOH
- 7 - Salicylic acid (5 mM) and 0.5 g/L SDS)
- 10 - Salicylic acid (1 mM) and Pentafluorobenzoic acid (2 mM)
- 21 - Silver nitrate (1 mM) in acidic pH (citric acid, pH 2.7)
- 24 - Silver nitrate (2 mM) in acidic pH (acetic acid, pH 2.7)
- 25 - Acetic acid (pH 2.7)
- 37 - Pentafluorobenzoic acid (5 mM)
- 38 - 2,6 - Dihydroxybenzoic acid (5 mM)
- 40 - Cinnamic acid (16.5 mM) and 35.5% EtOH
- 42 - Cinnamic acid (3.3 mM) and 7.1% EtOH
- 45 - 2-Fluorobenzoic acid (10 mM) and 2% EtOH
- 47 - UV-light irradiation of the whole plant for 3 hours
- 55 - Sodium fluoride (250 mM) and 10% EtOH

Fifteen treatments which elicited some of the highest levels of daidzein and genistein are shown in Figures 1 and 2 for simplicity. Some of the above-elicitors induced mild to moderate phytotoxicity in the treated plants. All compounds used in the mixtures produced significant levels of daidzein and genistein, when applied alone. However, combinations of

various elicitors shown in Figures 1 and 2 usually produced higher levels of target compounds. Other compounds used as elicitors, such as yeast extract, laminarin, SDS, jasmonic acid, methyl jasmonate, okadaic acid, polygalacturonic acid, 1-phosphatidic acid, polyethylene glycol, hydrogen peroxide, paraquat, calyculin A, 1-amino butyrate, eicosapentanoic acid, arachidonic acid, glutathione, ascorbic acid, and some heavy metals (nickel, copper, lead) showed lower degree of elicitation of the target compounds.

Various plants were grown hydroponically as previous described and secreted compounds (root exudates) were collected in distilled water with and without an elicitor(s).

Figure 3 is an HPLC profile of compounds recovered from the root exudates with UV detection at 251.8 nm. Most compounds were not identified, however, as shown in Figure 3 the following designations in Figure 3 were positively or putatively identified.

1. Positively identified Daidzein
2. Positively identified Genistein
- A. Putatively identified Nicotine
- B. Putatively identified at (5-O-methyl-genistein) *Lupin luteus* roots were not elicited; *Lupinus polyphyllus* roots were elicited with 2 mM Salicylic acid in 2% Ethanol; all other species were elicited with 0.3 M Acetic acid, pH 2.7.

Figure 4 demonstrates the diversity of compounds excreted from the roots of one plant species (*Lupinus luteus*) treated with different elicitors. (Note the large differences in the HPLC profiles of root exudates produced by different elicitors) UV detection at 251.8 nm.

Elicitor treatments:

control - No treatment

I - Treatment with 2 mM Salicylic acid in 2% Ethanol

II - Treatment with 0.3 M Acetic acid, pH - 2.7

### III - Treatment with 2 mM AgNO<sub>3</sub>

#### IV - Treatment with 7.5 mM Arachidonic acid

V - Treatment with 5 mM Jasmonic acid

Most compounds were not identified. Those positively or putatively identified are:

- 1 - Putatively identified as 5-O-Methyl-genistein  
G - Positively identified as Genistein

SA - Salicylic acid

Figures 5, 6, 7 and 8 are HPLC profiles of the diversity of compounds exuded or leached from the roots of *Brassica juncea*, *Datura metel*, *Lupinus polyphyllus* and *Melilotus medicaginoides*, respectively, treated with different elicitors. UV detection was at 254 nm. No compound was identified.

#### **Example 4. Root Extraction**

The complete root systems of all plants from each tray were excised, drained and weighed. Up to 30 g of the root systems (fresh weight) were sampled and stored at -20 °C. The root tissue was homogenized in a laboratory blender (Model 31BL91, Waring, New Hartford, CT) in 2 volumes H<sub>2</sub>O for approximately 30 sec. The homogenate was transferred to a 150 mL Corex tube (Corning, Inc., Corning, NY) and a two-phase extraction was carried out by adding 2 volumes of ethyl acetate (EA) and shaking the sealed tube for 30 min at 200 rpm (Shaker Model PR70, Hoefer Scientific Instrument, San Francisco, CA). The tubes were then centrifuged (Model Avanti J-25, Rotor No. JA-14, Beckman Instrument Inc., Palo Alto, CA) for 10 min at 4000 x g, in order to form a clear EA layer in the upper phase. The two-phase extraction was repeated with an additional single volume of EA. Following two extractions, the EA extracts were combined and placed in the fume hood until the EA volume was reduced to approximately half.

The EA extract was divided into disposable glass tubes in proportion to the weight of the extracted roots, so that each tube contained the extracts equivalent to at least 5 g root tissue. The EA extract was evaporated in a speed vac (Model AES2010, Savant Instruments, Inc., Farmingdale, NY), the tubes were sealed and stored at 20 °C. The H<sub>2</sub>O phase, containing the root tissue and some EA residues, was filtered, pressed through a 70 µm nylon mesh (Spectra/Mesh Nylon Filters, Spectrum, Houston, TX) and placed in a 125 mL separatory funnel until the lower water phase separated from the upper layer (approximately 30 min). The water layer was decanted into 50 mL polypropylene disposable tubes and centrifuged for 30 min at 4000 x g (Rotor No. JS-4.0, Beckman Instrument Inc.). The supernatant was divided into 60 mL glass bottles in proportion to the weight of the extracted root tissue (extract equivalent of 5 g of root tissue per bottle), freeze dried overnight (Genesis SQ12, Virtis, Gardiner, NY) and stored at -20 °C.

The remaining root tissue was further extracted with 2 volumes methanol (MeOH)/CH<sub>2</sub>Cl<sub>2</sub> (1:3), shaken for 30 min at 200 rpm, filtered and pressed through 70 µm fluorocarbon mesh (Spectra/Mesh Fluorocarbon Filters, Spectrum). The filtrate was transferred to a separatory funnel until the lower MeOH/CH<sub>2</sub>Cl<sub>2</sub> phase became clear (up to 30 min). The MeOH/CH<sub>2</sub>Cl<sub>2</sub> extract was then divided into disposable glass tubes (equivalent of 5 g root tissue per tube), dried in a speed vac and stored in a similar manner to the EA extracts.

#### **Example 5. High-pressure liquid chromatography (HPLC) analysis of extracted products**

The chromatography separation of extracted products was performed with an HPLC-system consisting of Waters 996 Photodiode Array Detector (PDA) with usable UV range from 190 to 800 nm; a Waters 717 plus autosampler; two Beckman 110B solvent Delivery Modules, connected with a Beckman System Organizer (mixer) and a Beckman System Gold Analog Interface Module 406. The Beckman solvent delivery system was controlled by a NEC PC-8300 computer. Chromatography and spectral data was managed by Waters Millennium chromatography software, version 2.10, using a NEC image 466es computer. All hardware components, except the solvent delivery system, were connected through a standard IEEE communication system. Compounds were separated on a Waters Nova Pak® C-18 reverse phase column, 3.9 x 150 mm, 60 Å pore size, and 4 µm particle size.

The mobile phase consisted of two components: Solvent A - 0.5% ACS grade acetic acid in double distilled water, pH 3-3.5; and Solvent B - acetonitrile. Prior to use, each batch of solvent A was degassed under vacuum and ultrasonication for 5 minutes.

The mobile phase flow was adjusted to 1 mL/min, and a gradient mode of separation was used for all separations. The gradient profile was as follows:

- 0 - 20 min 0% B - 100% B;
- 20 - 22 min 100% B;
- 22 - 25 min 100% B - 0% B;
- 25 - 33 min 0% B (column equilibration for next injection).

Compounds were detected with PDA detector within the wavelength range of 200 to 400 nm or with Waters Thermabeam™ Mass Detector. The column temperature was ambient.

All plants were grown hydroponically and treated with an elicitor, as described in Example 1. The roots were harvested and subjected to an extraction procedure as described in Example 4. The accompanying drawings are HPLC profiles (obtained as in Example 5) of chemicals recovered from the extracts, which extracts are recovered from roots harvested from the plants treated with elicitors described in the drawings.

### **Example 6: Bioassay of Leaf and Root Exudates**

A. Preparation of cuticular washings: Leaves from plants were contacted with 5 mL of solvent (methylene chloride) contained in plastic sandwich bags (quart size, 7 in x 8 in) as containers. To standardize the cuticular wash concentration by relating it to the used leaf surface from which it was taken, approximately 60 cm<sup>2</sup> of leaf surface were used. Sandwich bags with zippers were used to guarantee that the surface of leaf was totally moisturized with solvent. To facilitate the removal of cuticular compounds the bag containing a leaf and the solvent was shaken for approximately 20-40 seconds. The end of bag was cut and the content removed into 20 mL scintillation vials, and closed with Teflon or foil faced liner screw caps and stored in refrigerator. Alternatively, the solvent containing the cuticular washings can be dried inside the scintillation vial before cold storage.

B. Preparation of bacteria and fungus suspensions: six different organisms were used for antibacterial and antifungal screening: 1) *Escherichia coli* K-12. F, prototrophic Str.; 2) *Staphylococcus aureus* subsp. *Aureus*; 3) *Pseudomonas aeruginosa*; 4) *Saccharomyces cerevisiae*; 5) *Aspergillus flavus*; and 6) *Penicillium nigra*. Bacteria (*Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*) were maintained on solid agar media (LB Agar, Miller). Before screening, bacteria was transferred into liquid media and cultivated for 12 hours at 37 °C on shaker with a cell density 105-6. *Saccharomyces cerevisiae* (yeast), *Aspergillus flavus* and *Penicillium nigra* were cultivated on potato dextrose media. Before treatment yeast cells were transferred into liquid media and cultivated for 48 hours at 30 °C on a shaker. The spores of *Aspergillus flavus* were washed

with distilled water from fungus surface grown in Petri dish and resuspended in fresh distilled water.

The standard method used to determine *in vitro* antibacterial and antifungal activity of leaf surface cuticular washings consisted of testing suspension of microorganisms and spores of fungus for growth inhibition in the presence of washings. The antibacterial and antifungal activity was indicated by 30% or more, growth reduction of cells/spores in the presence of cuticular washings. All samples were plated in 3 replicates. One mL of nutrient media was put in each well in 24 well plates. Thereafter, 10  $\mu$ L of methylene chloride solution containing cuticular washings were placed on agar surface and after the drop dried (2-3 min) 30  $\mu$ L of microorganism suspension or fungus spores were plated on top of agar and equally distributed throughout the surface. After 24 hours of incubation at +30 °C, the plates were examined for the presence/absence of activity. To test antimicrobial/antifungal activity of root exudates, cell suspension was plated and spread on the agar surface into each of 24 well plates. Using a 5 mL Eppendorf pipet tip attached to a vacuum line, a hole was made in the center of each well and 20  $\mu$ L of exudate dissolved in water (5 mg/300 mL) gently poured into the hole. The following elicitors were employed: methyl salicylate, methyl jasmonate, silver nitrate, acetic acid, and chitosan.

To harness the vast and largely unexplored diversity of biological natural products exuded by plant roots, an efficient method for collecting root exudates from various plants was developed. The method was based on a modified hydroponic technology, which allowed maintaining plant roots in water or diluted nutrient solution followed by analysis of compounds exuded from roots.

The seeds of cultivated and wild species obtained from the commercial seed companies or botanical gardens were germinated in a greenhouse inside a 0.9 cm in diameter, 0.5 cm deep well cut into Grodan rockwool cubes (3.4 cm width x 3.4 cm depth x 3.7 cm height). Rockwool cubes were placed inside standard greenhouse plastic trays (dimensions 52 cm width x 25 cm depth x 7 cm height) and watered with an overhead misting system. Seeds were allowed to germinate for 3-6 days until the roots started to emerge from the bottom of the rockwool cube.

After germination, the cubes containing the seedlings were inserted into the center of a Styrofoam ring with an inside diameter 3.2 cm, outside diameter 8.2 cm and 2.5 cm thickness. The ring was floated on the surface of 400-800 mL of hydroponic nutrient

solution (2 g/L Hydro-Sol [Scotts-Sierra Horticultural products Comp., Marysville, OH] supplemented with 1.2 g/L  $\text{Ca}[\text{NO}_3]_2$ ) contained inside a light impermeable, high density polyethylene cylinder (9.0 cm in diameter, 16 cm in height).

Aeration was provided by shaking the cylinders at 50 rpm on a platform shaker (Labline Orbital Shaker, Model 3590). Seedlings were cultivated hydroponically in this system for 3 to 6 weeks with roots growing in a nutrient solution. Thereafter, the root system (average root dry weight  $0.1 \pm 0.05$  g) was removed from the nutrient solution and placed inside a 30 mL glass beaker, containing 10-20 mL of distilled water or distilled water supplemented with an elicitor. To prevent water loss from the plant canopy and drying of the collecting solution, plant shoots were covered with transparent plastic bags. After 24 hours, unless noted otherwise, a small sample from the root solution was removed and analyzed for the phytosecreted products. This system of hydroponic plant cultivation and exudate collection is referred to as the standard exudate collection system. Root exudates may also be freeze-dried and stored in the freezer at  $-20^\circ\text{C}$ . When needed, the exudate powder may be re-dissolved in water and used for screening or chemical analysis.

A total of 844 plant species root exudates elicited were prepared and tested against six-above mentioned microbial/fungal cultures (Table 2). The final concentration of exudates used in assay was 5 mg of dry exudate diluted in 300  $\mu\text{L}$  of distilled water. It has been found that great majority of material tested at this concentration did not affect adversely growth of the tested organisms. Figures 14, 15, 16, 19, 20, 21, and 22 demonstrate the results of the inhibition of the biological activity. A number of primary hits has been identified against all, but one (*Aspergillus*) microorganisms tested. The hit rate under the conditions used varied between 0% and 7.8% (Table 1). It is noteworthy that majority of the hits come from exudates from elicited roots. The unusually high proportion of hits in the materials elicited by silver may also be partially explained by the toxic effects of silver on a given microorganism.

**Table 1. Frequency of Antimicrobial Effects of Root Exudates**

Target Organism	Number of Hits	Hit Rate (%)
<i>Escherichia coli</i>	23 (884) <sup>1</sup>	2.6
<i>Staphylococcus aureus</i>	34 (884)	3.8

<i>Pseudomonas aeruginosa</i>	8 (102)	7.8
<i>Aspergillus flavus</i>	0 (510)	0
<i>Penicillium nigra</i>	4 (102)	3.9
<i>Saccharomyces cerevisiae</i>	6 (718)	0.8

Number in parenthesis indicate total number of root exudate samples tested for a particular microorganism.

The list of plant species from which root exudates show antimicrobial/antifungal activity are shown below in Table 2. The strengths of the activity is denoted by the number of "\*", with a larger number of "\*" referring to greater activity. As used in the table, the following abbreviations are intended to represent:

A.F. - *Aspergillus flavus*

E.c. - *Escherichia coli*

S.a. - *Staphylococcus aureus*

S.c. - *Saccharomyces cerevisiae*

P.n. - *Penicillium nigra*

P.a. - *Pseudomonas aeruginosa*

Acetic a. - acetic acid

Table 2. Root Exudates Showing Antimicrobial/Antifungal Activity

	I.D. #	Plant Name	Elicitor	A.f	E.c.	S.a.	S.c.	P.n.	P.a
1	845	<i>Atropa belladonna</i>	Silver			*			
2	857	<i>Erythrina glabelliformis</i>	Silver			*			
3	949	<i>Ipomoea tricolor</i>	Silver			*			
4	1363	<i>Erythrina galli</i>	Silver		*	*			
5	1475	<i>Celosia cristata</i>	Acetic a.		*	*			
6	1501	<i>Gallium spurium</i>	Acetic a.			*			
7	1513	<i>Laurus nobilis</i>	Silver		*	*			
8	1563	<i>Vitis labrusca</i>	Control		*				
9	1585	<i>Gratiola officinalis</i>	Control			*			



10	1617	Symphytum officinalis	Silver	*				
11	1645	Hosta fortunea	Acetic a.			*		
12	1649	Cassia hebecarpa	Control		*			
13	1659	Thalictrum flavum	Acetic a.	*	*			
14	1671	Scutellaria altissima	Silver	*	*			
15	1681	Portulacca oleracea	Silver			*		
16	1683	Portulacca oleracea	Chitosan			*		
17	1685	Portulacca oleracea	Meta			*		
18	1691	Scutellaria cretica	Silver		*			
19	1695	Physalis ixocarpa	Silver		*			
20	1757	Geum fourieri	Control			*		
21	1791	Gentiana tibetica		*	*			
22	1875	Linum hirsutum	Acetic a.			*		
23	1879	Aconitum napellus	Acetic a.					
24	1881	Aconitum napellus	Silver	*	*			
25	1887	Podophyllum aunodii	Silver					
26	1897	Thymus cretaceus	Silver				*	
27	1913	Hosta fortunea	Silver	***	***		*	
28	1975	Hosta fortunea	Chitosan	**	***			
29	1985	Carlina acaulis	Silver	*	***			
30	2003	Chamaechrista fasciculata	Silver	**	***		*	
31	2013	Pinus pinea	Silver	**	***		**	
32	2043	Peganum harmala	Silver		*			
33	2053	Tamarindus indica	Silver	*	***			
34	2063	Carica papaya	Silver	*	***			
35	2111	Cistus incanus	Control	***	**			
36	2161	Capparis inermis	Silver	***	***			** *
37	2177	Cupressus lusitanica	Silver	**	***			** *
38	2133	Diopiros khaki	Acetic a.		**			

39	2135	Diopiros khaki	Silver		**	***			**
40	2137	Diopiros khaki	Chitosan			**			
41	2145	Eryngium campestre	Silver		**	**			**
42	2255	Aesculus woerlitzienis	Silver		***	***			**
43	2265	Aesculus hippocastanum	Silver		***	***			**
44	2299	Cupressis sempervirens	Silver			***			
45	2315	Celtis occidentalis	Silver		**	***			
46	2325	Calycanthus floridus	Silver		**	**			
47	2335	Chinionanthus praecox	Control		**	**			
48	2345	Clematis manschurica	Silver		**	**			
49	2377	Liatris spicata	Silver			*			
50	2379	Liatris spicata	Silver			*			
51	2407	Cladium mariscus	Silver		**	***			
52	2425	Lablab purpureus	Silver			**			
53	2455	Campanula carpatica	Silver			*			
54	2443	Chilopsis linearis	Silver			*			
55	2461	Thuja occidentalis	Silver		**	**			
56	2479	Cosmos sulphureus	Silver		**	**	**		
57	2489	Cunningamia lanceolata	Silver		**	**	**		
58	2503	Euptelea pleiosperma	Silver			**			
59	2535	Juglans regia	Acetic a.		**	**			

A total of 400 plant species cuticular washings has been prepared and tested against six-above mentioned microbial/fungal cultures and the amount of cuticular washings used for each well was 10  $\mu$ L. It has been found that a majority of materials tested at this concentration did not affect adversely growth of the tested organisms. However, a significant percentage of cuticular washings showed antimicrobial activity. Figures 20, 21, 26, 27, 28, 29, 30, and 31 demonstrate the results of this activity. A number of strong antimicrobial hits have been identified for Escherichia coli, Staphylococcus aureus and Saccharomyces

cerevisiae Table 3. The hit rate among cuticular washings from different plant species varies from 0.5 to 5.0 % depending on the microorganism.

Table 3. Plant Species of which Leaf Surface Cuticular Washings Show Strong Antifungal or Antimicrobial Activity

	Sample Identification	Plant name	S. a.	E.c.	S.c.
1	125	Taxodium distichum	x		
2	133	Grevillea robusta	x		
3	136	Betula pendula	x		

4	171	Anthurium elegans	x		
5	198	Foenix zeulinica		x	x
6	216	Oreopanax capitate	x	x	
7	229	Eucalyptus rudis	x	x	x
8	248	Betula nigra	x		

9	274	<i>Paeonia dahurica</i>			
10	276	<i>Betula alba</i>	x	x	
11	294	<i>Thalictrum</i> sp.	x	x	
12	302	<i>Agrimonia eupatori</i>	x		
13	355	<i>Salix babalinica</i>	x		

14	377	Cerasus janonica			
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#### Example 7: Sniffing Test of Root Exudates and Cuticular Washings

The assay was done by thawing a frozen sample to room temperature, opening a glass vial containing a sample, sniffing it, and immediately marking the results. Samples are stored frozen at -20°C in tightly sealed glass vials (5 mL vials for cuticular washings and 20 mL vials for root exudates). The amount of root exudates in each vial ranges from 5 to 500 mg. The amounts of cuticular compounds in each vial ranges from 5 to 100 mg.

As demonstrated by Tables 4 and 5, a significant proportion of samples have a strong fragrance. There are 36 out of 100 samples of exudates and 20 out of 100 tested cuticular washings with strong fragrance. Root exudates were treated with Acetate 03 - 0.1% acetic acid, AgNO3 02 - 0.5 mM Ag(NO<sub>3</sub>)<sub>2</sub>, Chito 02 - 0.1% chitosan, water, HSL 01 - 200 µM N-hexanoyl homoerinelactone, and MeJa 03 - 100 µM methyl jasmonate. In Table 4 and 5, smell was rated by the scientist as follows: 0 is no smell; 1 is light smell; 2 is medium smell; and 3 is strong smell.

Table 4. Sniffing Test on Root Exudates

Sample	Rating	Family	Genus	Species	Treatment
1199	3	Solanaceae	Hyoscyamus	niger	Acetate 03
1201	3	Solanaceae	Hyoscyamus	niger	AgNO3 02
1203	2	Solanaceae	Hyoscyamus	niger	Chito 02
1205	3	Fabaceae	Genista	tinctoria	Acetate 3
1207	2	Fabaceae	Genista	tinctoria	AgNO3 02
1209	2	Fabaceae	Cicer	arietinum	control 01

1211	3	Fabaceae	Cicer	arietinum	Acetate 03
1213	3	Fabaceae	Cicer	arietinum	AgN03 02
1215	3	Fabaceae	Cicer	arietinum	Chito 02
1217	1	Fabaceae	Cicer	arietinum	HSL 01
1219	2	Fabaceae	Thermopsis	fabacea	HSL 01
1221	3	Fabaceae	Thermopsis	fabacea	acetate 03
1223	2	Cucurbitaceae	Trichosanthes	kirilowii	control 01
1225	3	Cucurbitaceae	Trichosanthes	kirilowii	acetate 03
1227	1	Cucurbitaceae	Trichosanthes	kirilowii	HSL 01
1229	2	Cucurbitaceae	Trichosanthes	kirilown	Chito 02
1233	0	Asteraceae	Xanthium	sibiricum	HSL 01
1235	3	Solanaceae	Brugmansia	suaevolens	AgN03 02
1237	0	Solanaceae	Brugmansia	suaevolens	HSLOI
1239	1	Asteraceae	Eclipta	alba	control 01
1241	3	Asteraceae	Eclipta	alba	Acetate 03
1243	2	Asteraceae	Eclipta	alba	AgN03 02
1245	0	Asteraceae	Eclipta.	alba	Chito
1247	3	Asteraceae	Aremisia	absinthium	control 01
1249	3	Asteraceae	Artemisia	absinthium	Acetate 3
1251	2	Asteraceae	Artemisia	absinthium	AgN03 02
1253	0	Asteraceae	Artemisia	absinthium	Chito 02
1255	1	Asteraceae	Silybum	marianum	control 0 1
1257	2	Asteraceae	Silybum	marianum	Acetate 03

1259	3	Asteraceae	Silybum	marianum	AgN03 02
1261	3	Asteraceae	Silybum	marianum	Chito 02
1263	2	Asteraceae	Silybum	marianum	MeJa 03
1267	3	Apiaceae	Cnidium	monnieri	Acetate 02
1269	2	Apiaceae	Cnidium	monnieri	AgN03 02
1271	2	Apiaceae	Cnidium	monnieri	Chito 02
1275	2	Apiaceae	Cnidium	monnieri	HSL 01
1277	3	Solanaceae	Brugmansia	suaevolens	control 01
1279	2	Solanaceae	Brugmansia	suaevolens	Acetate 03
1281	1	Clusiaceae	Hypericum	perforatum	control 0 1
1283	2	Clusiaceae	Hypericum	perforatum	Acetate 03
1285	1	Clusiaceae	Hypericum	perforatum	AgN03 02
1287	1	Clusiaceae	Hypericum	perforatum	Chito 02
1289	3	Clusiaceae	Hypericum	perforatum	MeJa 03
1291	3	Boraginaceae	Anchusa	officinalis	control 01
1293	2	Boraginaceae	Anchusa	officinalis	Acetate 03
1297	3	Asteraceae	Xanthium	sibiricum	AgN03 02
1299	3	Zygophyllaceae	Larrea	tridentata	control 01
1301	3	Zygophyllaceae	Larrea	tridentata	Acetate 03
1303	2	Zygophyllaceae	Larrea	tridentata	AgN03 02
1305	0	Zygophyllaceae	Larrea	tridentata	Chito 02
1307	2	Zygophyllaceae	Larrea	tridentata	MeJa 03
1309	2	Lamiaceae	Scutellaria	baicalensis	control 01





1359	0	Fabaceae	Erythrina	christa-calli	control 01
1361	3	Fabaceae	Erythrina	christa-galli	Acetate 03
1363	2	Fabaceae	Erythrina	christa-cralli	AgNO3 02
1365	1	Fabaceae	Erythrina	christa-galli	Chito 02
1367	0	Fabaceae	Erythrina	christa-galli	HSL 01
1369	3	Ranunculaceae	Aquilegia	vulgaris	control 01
1371	1	Ranunculaceae	Aquilegia	vulgaris	Acetate 03
1373	1	Ranunculaceae	Aquilegia	vulgaris	AgNO3 02
1375	1	Ranunculaceae	Aquilegia	vulgaris	Chito 02
1377	2	Ranunculaceae	Aquilegia	vulgaris	MeJa 03
1379	3	Lamiaceae	Leonurus	sibiricus	control 01
1381	3	Lamiaceae	Leonurus	sibiricus	Acetate 03
1383	2	Lamiaceae	Leonurus	sibiricus	AgNO302
1385	0	Lamiaceae	Leonurus	sibiricus	Chito 02
1387	1	Lamiaceae	Leonurus	sibiricus	MeJa 03
1395	1	Ephedraceae	Ephedra	nevadensis	Acetate 03
1397	3	Convolvulaceae	Ipomoea	purpurea	control 01
1399	3	Convolvulaceae	Ipomoea	purpurea	Acetate 03
1401	2	Convolvulaceae	Ipomoea	purpurea	AgNO3 02
1403	2	Convolvulaceae	Ipomoea	purpurea	Chito 02
1405	3	Convolvulaceae	Ipomoea	purpurea	MeJa 03
1407	3	Amaranthaceae	Cyathula	officinalis	Acetate 03
1409	3	Asteraceae	Xanthium	sibiricum	Acetate 03
1413	3	Fabaceae	Tephrosia	grandiflora	Acetate 03

Table 5. Sniffing Test on Cuticular Washings

Sample #	Rating	Family	Genus	Species
wx 201	1	Rutaceae	Murrays	exotica
wx 202	2	Araliaceae	Trevesia	sungaica
wx 203	1	Verbenaceae	Clerodendrum	speciosissimum
wx 204	0	Euphorbiaceae	Acalypha	hispida



wx 236	1	Rosaceae	Geum	macrophyllum
wx 237	2	Magnoliaceae	Magnolia	kobus
wx 238	3	Apocynaceae	Vinca	minor
wx 239	0	Liliaceae	Convallaria	majalis
wx 240	2	Betulaceae	Corvulus	avellana
wx 241	2	Berberidaceae	Berberis	sp.
wx 242	2	Rosaceae	Rosa	multiflora
wx 243	1	Betulaceae	Ostrya	carpinifolia
wx 244	1	Betulaceae	Ostrya	connogea
wx 245	1	Fagaceae	Quercus	rubra
wx 246	2	Magnoliaceae	Liriodendron	tulipifera
wx 247	1	Rosaceae	Sorbus	aucuparia
wx 248	3	Betulaceae	Betula	nigra
wx 249	3	Betulaceae	Betula	nigra
wx 250	2	Fagaceae	Castanea	sativa
wx 251	0	Saxofragaceae	Bergenia	crussifolia
wx 252	3	Asteraceae	Artemisia	dracunculus
wx 253	3	Rutaceae	Ruta	graveolens
wx 254	3	Fragaceae	Quercus	nigra
wx 255	2	Schisandraceae	Schisandra	chinensis
wx 256	3	Betulaceae	Betula	alba
wx 257	2	Caprifoliaceae	Sambucus	nigra
wx 258	3	Actinidiaceae	Actinidia	colonicta
wx 259	2	Paoniaceae	Paeonia	lactiflora
wx 260	1	Paoniaceae	Paeonia	suffructicosa
wx 261	0	Fragaceae	Quercus	imbricaria
wx 262	1	Indaceae	Iris	pallida
wx 263	2	Portulacaceae	Portulacca	oleracea
wx 264	2	Polygonaceae	Polygonum	aviculare
wx 265	0	Iridaceae	Iris	pseudacorus
wx 266	1	Liliaceae	Allium	nutans



wx 298	3	Menispermaceae	Menispermum	dauricum
wx 299	3	Nyctaginaceae	Oxybaphus	nyctagineus
wx 300	2	Brassicaceae	Armoracia	rusticana

### Example 8: Anti-microbial Activity Elicited by Acetic Acid

Anti-microbial activity was assessed using a whole-cell growth inhibition bioassay. More particularly, anti-fungal activity was assessed using *Saccharomyces cerevisiae* and *Aspergillus niger*; anti-bacterial activity was measured using *Staphylococcus aureus* (gram-positive) and *Escherichia coli* and *Pseudomonas aeruginosa* (gram-negative). To ensure that positive results reflected true anti-microbial activity, these test cells were subjected to the inhibition assay in the presence of acetic acid and in the absence of the elicitor (*i.e.*, control).

Plants were germinated and grown, extracts were prepared, and bioassays were conducted as described above (see Examples 1, 2 and 6 in particular). Example 6 and Table 2 also indicate the wide variety of plants whose extracts were subjected to testing. In brief, plants were grown as described in Example 1, above. Following growth, plants were removed from the nutrient solution and placed in either distilled water (control) or in 0.1% (v/v) acetic acid, typically for 24 hours (see Example 1). Extracts of plant roots were generally prepared according to the protocol described in Example 4. In particular, plant roots (approximately 1 - 5 g fresh weight) were harvested and freeze-dried, followed by an extraction with 20 ml of 80% (v/v) methanol per gram of lyophilized roots at room temperature for 48 hours. The methanol-root compositions were then centrifuged and the supernatant was decanted. The extract was formed by drying the supernatant by evaporation. Typically, 100-120  $\mu$ g of extract were used in each bioassay.

Microbial organisms used in the bioassays were grown as described (see Example 1). To conduct the bioassays, 24-well microtiter plates were used with growing microbes

exposed to an extract (resulting from elicitation with acetic acid or a control extract resulting from exposure of the plant to water) or to a known inhibitor of microbe growth (kanamycin for bacteria; ketoconazole for fungi). In a fixed number of wells per plate, microbes (either bacteria, or fungi) were grown without presence of an extract and known antibiotic. The growth in these wells was used for comparison in assessing the growth inhibition potential of the plant extracts tested.

Table 6. Anti-microbial activity of extracts elicited with acetic acid.

Family	Genus	species	treatment	Staph	Ecoli	Saccheer	Aspnig	Pseudom
Agavaceae	Agave	chrysantha	control 01			4,4,5		
Agavaceae	Agave	chrysantha	Acetate 03			4,4,5		
Agavaceae	Agave	neomexicana	control 01			4,4,5		
Agavaceae	Agave	neomexicana	Acetate 03			4,4,5		
Agavaceae	Yucca	baccata	control 01			5,0		
Agavaceae	Yucca	baccata	Acetate 03			5,0	3,2	
Agavaceae	Yucca	whipplei	control 01					
Agavaceae	Yucca	whipplei	Acetate 03			5		
Alzooaceae	Aloinopsis	spathulata	control 01	2,0,1	1,0,1			
Alzooaceae	Aloinopsis	spathulata	Acetate 03	2,0,1	1,0,1			
Alzooaceae	Hypertelis	salsoides	control 01					
Alzooaceae	Hypertelis	salsoides	Acetate 03	3				
Alzooaceae	Allium	ampeloprasum	control 01			5,5,5		
Alzooaceae	Allium	ampeloprasum	Acetate 03			5,5,5		
Amaranthaceae	Amaranthus	hypochondriacus	control 01	2				
Amaranthaceae	Amaranthus	hypochondriacus	Acetate 03	2				
Amaranthaceae	Amaranthus	powellii	control 01					
Amaranthaceae	Amaranthus	powellii	Acetate 03			3		
Amaranthaceae	Amaranthus	retroflexus	control 01					
Amaranthaceae	Amaranthus	retroflexus	Acetate 03	2		2,0,0		
Amaryllidaceae	Agapanthus	africanus	control 01			5		
Amaryllidaceae	Agapanthus	africanus	Acetate 03			5	5	
Anacardiaceae	Cotinus	coggygia	control 01	2	3	3		
Anacardiaceae	Cotinus	coggygia	Acetate 03	2				
Anacardiaceae	Malosma	laurina	control 01					
Anacardiaceae	Malosma	laurina	Acetate 03		2			
Anacardiaceae	Rhus	integrifolia	control 01	3				
Anacardiaceae	Rhus	integrifolia	Acetate 03	3				
Anacardiaceae	Rhus	typhina	control 01	3				
Anacardiaceae	Rhus	typhina	Acetate 03	3				
Anacardiaceae	Schinus	terebinthifolius	control 01					
Anacardiaceae	Schinus	terebinthifolius	Acetate 03	3				
Apiaceae	Actinotus	helianthi	control 01	4,4	2,0	2,2	2,2	1,0



Apiaceae	Adnitus	helianthi	Acetate 03	3,0	1,0			1,0
Apiaceae	Amni	visnaga	control 01	4				
Apiaceae	Amni	visnaga	Acetate 03	3				
Apiaceae	Angelica	archangelica	control 01	2				
Apiaceae	Angelica	archangelica	Acetate 03	2		3		
Apiaceae	Anthriscus	cerefolium	control 01					
Apiaceae	Anthriscus	cerefolium	Acetate 03	2				
Apiaceae	Anthriscus	cerefolium	control 01	4		3	3	2
Apiaceae	Anthriscus	cerefolium	Acetate 03	4		4		1,0
Apiaceae	Bunium	bulbocastanum	control 01	5,5	1,0	4,4		1,0
Apiaceae	Bunium	bulbocastanum	Acetate 03	4,4		3,3		1,2
Apiaceae	Bupleurum	aureum	control 01	3,4	2,0	2,0		2,0
Apiaceae	Bupleurum	aureum	Acetate 03	2,3	2,2	2,0		2,0
Apiaceae	Carum	roseburghianum	control 01					
Apiaceae	Carum	roseburghianum	Acetate 03	3		3		
Apiaceae	Crithmum	maritimum	control 01	3,3c				
Apiaceae	Crithmum	maritimum	Acetate 03	3,4c			4,4	
Apiaceae	Cryptotaenia	japonica	control 01	4,1,4		3,0,4		
Apiaceae	Cryptotaenia	japonica	Acetate 03	3,0,2				
Apiaceae	Cuminum	cuminum	control 01	2,0,0				
Apiaceae	Cuminum	cuminum	Acetate 03	1,0,0				
Apiaceae	Dasispermum	suffruticosum	control 01	4,4		2,0		3,3
Apiaceae	Dasispermum	suffruticosum	Acetate 03	4,4		2,4		4,4
Apiaceae	Ferula	communis	control 01	3c,3c				
Apiaceae	Ferula	communis	Acetate 03	3c,2				
Apiaceae	Ferula	communis	control 01					
Apiaceae	Ferula	communis	Acetate 03					
Apiaceae	Foeniculum	vulgare	control 01	4,5		2,2	2,2	
Apiaceae	Foeniculum	vulgare	Acetate 03	3,4		2,1		
Apiaceae	Foeniculum	vulgare	control 01			5		
Apiaceae	Libanotis	montana	control 01			3		
Apiaceae	Libanotis	montana	Acetate 03					
Apiaceae	Ligusticum	porteri	control 01		1,0,0			
Apiaceae	Ligusticum	porteri	Acetate 03	2,0,0	1,0,1	3,0,0		
Apiaceae	Petroselinum	crispum	control 01					
Apiaceae	Petroselinum	crispum	Acetate 03	3	2	4		
Apiaceae	Petroselinum	crispum	control 01					

Apiaceae	Petroselinum	crispum	Acetate 03				
Apiaceae	Petroselinum	crispum	control 01				
Apiaceae	Petroselinum	crispum	Acetate 03				
Apiaceae	Pimpinella	saxifraga	control 01	3			
Apiaceae	Pimpinella	saxifraga	Acetate 03	4			
Apiaceae	Sium	sisarum	control 01	3,4			
Apiaceae	Sium	sisarum	Acetate 03	5,4		2,0	3,3
Apiaceae	Sium	sisarum	control 01				1,1
Apiaceae	Smyrnium	olusatrum	Acetate 03	1,2			
Apiaceae	Smyrnium	olusatrum	control 01	5,4		1,3	3,3
Apiaceae	Steganothaenia	araliacea	Acetate 03	4,4		4,4	4,4
Apiaceae	Steganothaenia	araliacea	Acetate 03	4,4			3,0
Apiaceae	Tonilis	arvensis	Acetate 03	3c,3		2,0	1,2
Apiaceae	Trachymene	caerulea	control 01	1,4	2,0	1,2	2,2
Apiaceae	Trachymene	caerulea	Acetate 03	4,4	2,0	1,2	2,2
Apiaceae	Trachyspermum	ammi	control 01	4,4			
Apiaceae	Trachyspermum	ammi	Acetate 03	5,4		2,3	4,3
Apiaceae	Zizia	aptera	control 01	5,4	2,0	3,3	2,4
Apiaceae	Zizia	aptera	Acetate 03	4,4	2,0	3,2	2,3
Apiaceae	Zizia	aptera	control 01				
Apiaceae	Catharanthus	roseus	control 01				
Apiaceae	Catharanthus	roseus	control 01				
Apiaceae	Catharanthus	roseus	control 01				
Apiaceae	Catharanthus	roseus	Acetate 01				
Apiaceae	Catharanthus	roseus	Acetate 01				
Apiaceae	Catharanthus	roseus	control 01	1,0	3,0		
Apiaceae	Catharanthus	roseus	control 01		3,0		
Apiaceae	Catharanthus	roseus	Acetate 03				
Apiaceae	Catharanthus	roseus	control 01	3,2,2		2,0,0	
Apiaceae	Araucaria	araucana	Acetate 03	3,2,3		5	
Apiaceae	Araucaria	araucana	control 01			5	
Apiaceae	Phoenix	dactylifera	control 01			5	
Apiaceae	Phoenix	dactylifera	Acetate 03			3	2
Apiaceae	Phoenix	dactylifera	control 01			4	2
Apiaceae	Phoenix	dactylifera	Acetate 03			5	
Apiaceae	Trachycarpus	excelsus	control 01			5	
Apiaceae	Trachycarpus	excelsus	Acetate 03				
Asphodelaceae	Kniphofia	baurii	control 01				
Asphodelaceae	Kniphofia	baurii	Acetate 03	3			

Asteraceae	Achillea	filipendulina	control 01				
Asteraceae	Achillea	filipendulina	Acetate 03	3			
Asteraceae	Achillea	millefolium	control 01				
Asteraceae	Achillea	millefolium	control 01				
Asteraceae	Achillea	millefolium	Acetate 02				
Asteraceae	Achillea	millefolium	Acetate 02				
Asteraceae	Achillea	millefolium	control 01				
Asteraceae	Achillea	millefolium	Acetate 03	4			
Asteraceae	Achillea	millefolium	control 01				
Asteraceae	Achillea	millefolium	Acetate 03				
Asteraceae	Achillea	millefolium	control 01				
Asteraceae	Agoseris	grandiflora	Acetate 03	2,0	2,2		2,2
Asteraceae	Agoseris	grandiflora	control 01	2,0			
Asteraceae	Amellus	asteroides	Acetate 03	1,0	1,0		
Asteraceae	Amellus	asteroides	control 01				
Asteraceae	Anacyclus	pyrethrum	Acetate 03	4,3	2,2	1,1	3,2
Asteraceae	Anacyclus	pyrethrum	control 01			2,2	3,3
Asteraceae	Anthemis	nobilis	control 01				
Asteraceae	Anthemis	nobilis	Acetate 03				2
Asteraceae	Arctanthemum	arcticum	control 01	2,1			
Asteraceae	Arctanthemum	arcticum	Acetate 03	2,3			3,0
Asteraceae	Arctotheca	calendula	control 01				
Asteraceae	Arctotheca	calendula	Acetate 03	2,0			
Asteraceae	Argyranthemum	frutescens	control 01	1,1	1,0		
Asteraceae	Argyranthemum	frutescens	Acetate 03	2,0			
Asteraceae	Artemisia	absinthium	control 01				
Asteraceae	Artemisia	absinthium	Acetate 03	3			
Asteraceae	Artemisia	douglasiana	control 01				
Asteraceae	Artemisia	douglasiana	Acetate 03	3			
Asteraceae	Artemisia	dracunculifolia	control 01	2			
Asteraceae	Artemisia	dracunculifolia	Acetate 03	4			
Asteraceae	Artemisia	sukksdorfii	control 01				
Asteraceae	Artemisia	sukksdorfii	Acetate 03	4			
Asteraceae	Artemisia	sukksdorfii	control 01				
Asteraceae	Athanasia	orthoceras	control 01	2,0			

Asteraceae	Athanasia	grinnifolia	Acetate 03	1,3	1,1	3,3	
Asteraceae	Atractylodes	macrocephala	control 01				
Asteraceae	Atractylodes	macrocephala	Acetate 03	3			
Asteraceae	Baccharis	emoryi	control 01	3c,3c		4,2	
Asteraceae	Baccharis	emoryi	Acetate 03	3c,3c	2,2	2,3	3,2
Asteraceae	Berkheya	purpurea	control 01				2,3
Asteraceae	Berkheya	purpurea	Acetate 03				
Asteraceae	Boltonia	decurrens	control 01				
Asteraceae	Boltonia	decurrens	Acetate 03	2,2		1,0	
Asteraceae	Bracteantha	macrantha	control 01	1,1		2,0	1,0
Asteraceae	Bracteantha	macrantha	Acetate 03	3,3		2,2	1,0
Asteraceae	Bupthalmum	salicifolium	control 01	3,2	2,2	1,0	
Asteraceae	Bupthalmum	salicifolium	Acetate 03	4,4	2,2		
Asteraceae	Callistephus	chinensis	control 01		2,1	4,4	
Asteraceae	Callistephus	chinensis	Acetate 03	1,2		3,4	
Asteraceae	Carduncellus	multissimus	control 01	2,1	1,1		2,0
Asteraceae	Carduncellus	multissimus	Acetate 03	2,2			1,0
Asteraceae	Carthamus	tinctorius	control 01				1,1
Asteraceae	Carthamus	tinctorius	Acetate 03	5	3		
Asteraceae	Cassinia	aculeata	control 01	2,0	1,0		1,1
Asteraceae	Cassinia	aculeata	Acetate 03	3,3	1,1	2,3	1,1
Asteraceae	Centaurea	maculosa	control 01				
Asteraceae	Centaurea	maculosa	Acetate 03	2,1,2			
Asteraceae	Chamaemelum	nobile	control 01		3,0		
Asteraceae	Chamaemelum	nobile	Acetate 03		2,3		
Asteraceae	Chrysanthemum	coronarium	control 01			2	
Asteraceae	Chrysanthemum	coronarium	Acetate 03				
Asteraceae	Chrysanthemum	coronarium	control 01				
Asteraceae	Chrysanthemum	coronarium	Acetate 03				
Asteraceae	Chrysanthemum	leucanthemum	control 01				
Asteraceae	Chrysanthemum	leucanthemum	Acetate 03	4			
Asteraceae	Chrysanthemum	parthenium	control 01			5	
Asteraceae	Chrysanthemum	parthenium	Acetate 03	2			
Asteraceae	Chrysanthemum	nauseosus	control 01	1,0			2,3
Asteraceae	Chrysanthemum	nauseosus	Acetate 03	2,2			2,2
Asteraceae	Cicerbita	alpina	control 01				

Asteraceae	Cicerbita	alpina	Acetate 03	1,0				1,0
Asteraceae	Cirsium	vulgare	control 01		1,0			1,0
Asteraceae	Cirsium	vulgare	Acetate 03	4,3				1,0
Asteraceae	Cnicus	benedictus	control 01		2			
Asteraceae	Cnicus	benedictus	Acetate 03		1			
Asteraceae	Cnicus	benedictus	control 01	2				
Asteraceae	Cnicus	benedictus	Acetate 03	3	1			
Asteraceae	Coleostephus	myconis	control 01	2,0	2,0			3,0
Asteraceae	Coleostephus	myconis	Acetate 03	1,2				3,2
Asteraceae	Conoclinium	coelestinum	control 01	2,1	2,0	2,0		1,0
Asteraceae	Conoclinium	coelestinum	Acetate 03	2,2	3,2	1,0		1,0
Asteraceae	Coreopsis	tinctoria	control 01	3				
Asteraceae	Coreopsis	tinctoria	Acetate 03	3				
Asteraceae	Corethrogyne	californica	control 01					
Asteraceae	Corethrogyne	californica	Acetate 03	3				
Asteraceae	Doronicum	orientale	control 01	1,0				1,0
Asteraceae	Doronicum	orientale	Acetate 03	4,3	3,3		2,1	2,2
Asteraceae	Elephantopus	scaber	control 01			2,2		
Asteraceae	Elephantopus	scaber	Acetate 03		2,0			2,0
Asteraceae	Emilia	gocchiea	control 01			1,0	3,0	
Asteraceae	Emilia	gocchiea	Acetate 03	2,4c		1,2		2,0
Asteraceae	Encelia	californica	control 01	3,2	1,2		2,0	1,1
Asteraceae	Encelia	californica	Acetate 03	4,4		2,2	2,1	
Asteraceae	Eriophyllum	staechadiifolium	control 01					
Asteraceae	Eriophyllum	staechadiifolium	Acetate 03			4		
Asteraceae	Eupatorium	cannabinum	control 01					
Asteraceae	Eupatorium	cannabinum	Acetate 03		2,0,1	1		
Asteraceae	Eupatorium	maculatum	control 01			3,3,2		
Asteraceae	Eupatorium	maculatum	Acetate 03	2,2,2		4,3,2		
Asteraceae	Felicia	ameloides	control 01	2,1	2,2	1,0		2,0
Asteraceae	Felicia	ameloides	Acetate 03	2,4	2,4	4,0		4,3
Asteraceae	Felicia	echinata	control 01					
Asteraceae	Felicia	echinata	Acetate 03	4,0	2,2			
Asteraceae	Foveolina	tenella	control 01	1,2	2,3	2,2		2,0
Asteraceae	Foveolina	tenella	Acetate 03	2,3	2,0	3,2	2,2	2,2
Asteraceae	Gaillardia	aristata	control 01	4				

Asteraceae	Gallardia	aristata	Acetate 03	2				
Asteraceae	Galactites	tomentosa	control 01	4,1			2,2	
Asteraceae	Galactites	tomentosa	Acetate 03	4,4				3,3
Asteraceae	Geraea	viscida	control 01					
Asteraceae	Geraea	viscida	Acetate 03	4,3		2,0		1,0
Asteraceae	Gnaphalium	californicum	control 01					
Asteraceae	Gnaphalium	californicum	Acetate 03	4,4				3,2
Asteraceae	Heliopsis	helianthoides	control 01	1,2				4,0
Asteraceae	Heliopsis	helianthoides	Acetate 03	1,3		2,3		
Asteraceae	Heliopsis	helianthoides	control 01					1,0
Asteraceae	Helipterum	argyropsis	control 01					
Asteraceae	Helipterum	argyropsis	Acetate 03	1,0				
Asteraceae	Hemizonia	congesta	control 01			2,1	1,0	
Asteraceae	Hemizonia	congesta	Acetate 03	4,3		2,1	2,2	
Asteraceae	Heterolepis	aliena	control 01					1,1
Asteraceae	Heterolepis	aliena	Acetate 03					
Asteraceae	Heterolepis	aliena	Acetate 03	3,2				2,2
Asteraceae	Hulsea	heterochroma	control 01					2,2
Asteraceae	Hulsea	heterochroma	Acetate 03	3,3c		3,0	4,2	3,0
Asteraceae	Hulsea	parviflora	control 01					2,1
Asteraceae	Hymenolepis	parviflora	Acetate 03	2,2		2,2		2,0
Asteraceae	Hymenolepis	parviflora	control 01					1,0
Asteraceae	Inula	ensifolia	Acetate 03			1,0,1		
Asteraceae	Inula	ensifolia	control 01	2,0				
Asteraceae	Jurinea	mollis	Acetate 03	4,4				
Asteraceae	Jurinea	mollis	control 01					2,2
Asteraceae	Lasiospermum	bipinnatum	Acetate 03	1,3				2,3
Asteraceae	Lasiospermum	bipinnatum	control 01					
Asteraceae	Lasthenia	glabrata	Acetate 03					2
Asteraceae	Lasthenia	glabrata	control 01					
Asteraceae	Lasthenia	glabrata	Acetate 03					2
Asteraceae	Leontodon	autumnalis	control 01			2,2	3,2	
Asteraceae	Leontodon	autumnalis	Acetate 03				2,0	3,4
Asteraceae	Leucanthemopsis	alpina	control 01	2,2		2,0		1,0
Asteraceae	Leucanthemopsis	alpina	Acetate 03	2,3		1,1	1,0	1,2
Asteraceae	Leucanthemopsis	alpina	control 01					4,0
Asteraceae	Leucheria	cerberoa	control 01					
Asteraceae	Leucheria	cerberoa	Acetate 03	4,4		1,3	2,2	2,2
Asteraceae	Liatris	spicata	control 01					
Asteraceae	Liatris	spicata	Acetate 03				3,4,0	

Asteraceae	Madia	elegans	control 01	1,2				
Asteraceae	Madia	elegans	Acetate 03	4,4	1,1			2,0
Asteraceae	Malacothrix	californica	control 01	2,0				
Asteraceae	Malacothrix	californica	Acetate 03	2,4	2,1	1,1		1,1
Asteraceae	Matricaria	matricarioides	control 01			2,2		1,2
Asteraceae	Matricaria	matricarioides	Acetate 03	1,0	1,2			
Asteraceae	Neuolaena	lobata	control 01					
Asteraceae	Neuolaena	lobata	Acetate 03	4,5		2,4		
Asteraceae	Oldenburgia	grandis	control 01	2,0		3,1		2,1
Asteraceae	Oldenburgia	grandis	Acetate 03	1,2	2,2			2,1
Asteraceae	Oncosiphon	grandiflorum	control 01	3,0				
Asteraceae	Oncosiphon	grandiflorum	Acetate 03	3,0	1,2			
Asteraceae	Onopordum	acanthium	control 01		1,0			
Asteraceae	Onopordum	acanthium	Acetate 03	1,2	2,2	2,2		
Asteraceae	Phagnalon	saxatile	control 01					
Asteraceae	Phagnalon	saxatile	Acetate 03	2,2	2,0	1,0		
Asteraceae	Phymaspermum	acerosum	control 01	1,1	2,2			1,1
Asteraceae	Phymaspermum	acerosum	Acetate 03	3c,2	1,2			
Asteraceae	Picris	echioides	control 01	1,0	1,0			1,0
Asteraceae	Picris	echioides	Acetate 03		3,1			1,0
Asteraceae	Polymnia	uvellata	control 01		3,0	1,0		2,2
Asteraceae	Polymnia	uvellata	Acetate 03	4,4	2,0	2,3		2,2
Asteraceae	Porophyllum	ruderalis	control 01					
Asteraceae	Porophyllum	ruderalis	Acetate 03	5				
Asteraceae	Ptilostemon	after	control 01	4,4		2,2		3,0
Asteraceae	Ptilostemon	after	Acetate 03	4,4		1,2		4,0
Asteraceae	Pulicaria	dysenterica	control 01	2,0	3,2			1,0
Asteraceae	Pulicaria	dysenterica	Acetate 03	3,3		2,0		
Asteraceae	Pyrethrum	corymbosum	control 01					
Asteraceae	Pyrethrum	corymbosum	control 01					
Asteraceae	Pyrethrum	corymbosum	Acetate 03	2,2	2,1	3,2		3,2
Asteraceae	Saussurea	heteromala	control 01	3,0				1,0
Asteraceae	Saussurea	heteromala	Acetate 03	3,2				
Asteraceae	Scorzonera	hispanica	control 01	3,1	2,0			
Asteraceae	Scorzonera	hispanica	Acetate 03	2,1				1,1
Asteraceae	Senecio	cineraria	control 01					

Asteraceae	Senecio	cineraria	Acetate 03	3				
Asteraceae	Silybum	marianum	control 01					
Asteraceae	Silybum	marianum	Acetate 03	3,3,3		0,0,3		
Asteraceae	Sinacalia	tangutica	control 01		1,0			1,0
Asteraceae	Sinacalia	tangutica	Acetate 03	4,3		2,0	3,0	3,0
Asteraceae	Solidago	virgaurea	control 01					
Asteraceae	Solidago	virgaurea	Acetate 03	4		4		
Asteraceae	Solidago	virgaurea	control 01					
Asteraceae	Sonchus	oleraceus	control 01	2,2	3,1	3,2		2,0
Asteraceae	Sonchus	oleraceus	Acetate 03	4,4	2,2	2,2		2,1
Asteraceae	Stephanomeria	virgata	control 01	2,2	2,0	2,2	2,2	2,0
Asteraceae	Stephanomeria	virgata	Acetate 03	3,3,c	2,0	2,2	3,2	2,0
Asteraceae	Tanacetum	camphoratum	control 01		1,2			2,1
Asteraceae	Tanacetum	camphoratum	Acetate 03	1,0				1,2
Asteraceae	Tripleurospermum	inodorum	control 01		1,0			
Asteraceae	Tripleurospermum	inodorum	Acetate 03		3,2			
Asteraceae	Venegasia	carpesioides	control 01				4,0	2,0
Asteraceae	Venegasia	carpesioides	Acetate 03	4,3,c	2,0		3,3	
Asteraceae	Viguiera	lacinata	control 01	2,1	2,2			2,3
Asteraceae	Viguiera	lacinata	Acetate 03	3,c,3,c	2,1	3,4	2,2	2,2
Asteraceae	Wedelia	biflora	control 01	1,0				
Asteraceae	Wedelia	biflora	Acetate 03	2,3			2,0	
Asteraceae	Xanthisma	texanum	control 01	3,2				
Asteraceae	Xanthisma	texanum	Acetate 03	4,3				1,0
Asteraceae	Xanthium	strumarium	control 01			1,0,0		
Asteraceae	Xanthium	strumarium	Acetate 03					
Asteraceae	Xeranthemum	annuum	control 01	2,3,c		2,2		
Asteraceae	Xeranthemum	annuum	Acetate 03	3,3			3,0	
Berberidaceae	Berberis	thunbergii	control 01					
Berberidaceae	Berberis	thunbergii	Acetate 03	2,4,4		0,0,2		
Berberidaceae	Berberis	thunbergii	control 01					
Berberidaceae	Berberis	thunbergii	Acetate 03					
Berberidaceae	Nandina	domestica	control 01					
Berberidaceae	Nandina	domestica	control 01					
Berberidaceae	Nandina	domestica	Acetate 03	3				
Berberidaceae	Podophyllum	ernodii	control 01					



Berberidaceae	Podophyllum	ernodi	Acetate 03	2,0,3				
Berberidaceae	Podophyllum	hexandrum	control 01	3,2,3				
Berberidaceae	Podophyllum	hexandrum	Acetate 03	3,2,3				
Betulaceae	Carpinus	orientalis	control 01		1,0			2,1
Betulaceae	Carpinus	orientalis	Acetate 03					
Bignoniaceae	Chilopsis	linearis	control 01	1,0,1				
Bignoniaceae	Chilopsis	linearis	Acetate 03					
Bignoniaceae	Chilopsis	linearis	control 01					
Bignoniaceae	Chilopsis	linearis	Acetate 03					
Boraginaceae	Anchusa	officinalis	control 01					
Boraginaceae	Anchusa	officinalis	Acetate 03	1,0,3				
Boraginaceae	Myosotis	sylvatica	control 01					
Boraginaceae	Myosotis	sylvatica	Acetate 03	5				
Brassicaceae	Brassica	hirta	control 01	2,0,0	2,0,1			
Brassicaceae	Brassica	hirta	Acetate 03	1	2,0,2			
Brassicaceae	Brassica	oleracea	control 01					
Brassicaceae	Brassica	oleracea	control 01					
Brassicaceae	Brassica	oleracea	control 01					
Brassicaceae	Brassica	oleracea	Acetate 01					
Brassicaceae	Brassica	oleracea	Acetate 01					
Brassicaceae	Brassica	oleracea	control 01					
Brassicaceae	Brassica	oleracea	control 01					
Brassicaceae	Brassica	oleracea	control 01					
Brassicaceae	Brassica	oleracea	control 01					
Brassicaceae	Brassica	oleracea	Acetate 01					
Brassicaceae	Brassica	oleracea	Acetate 01					
Brassicaceae	Brassica	oleracea	control 01	1				
Brassicaceae	Brassica	oleracea	control 01					
Brassicaceae	Brassica	oleracea	Acetate 03		2			
Brassicaceae	Brassica	rapa	control 01					
Brassicaceae	Brassica	rapa	Acetate 03		3,1,0			
Brassicaceae	Brassica	rapa	control 01					
Brassicaceae	Brassica	rapa	Acetate 03					
Brassicaceae	Brassica	rapa	control 01					
Brassicaceae	Brassica	rapa	Acetate 03					
Brassicaceae	Brassica	rapa	control 01					
Brassicaceae	Brassica	rapa	Acetate 03	3	3			
Brassicaceae	iberis	coronaria	control 01					

Brassicaceae	Iberis	coronaria	Acetate 03	control 01	1		
Brassicaceae	Iberis	umbellata	Acetate 03	control 01	2	3	
Brassicaceae	Isatis	tinctoria	Acetate 03	control 01	2		
Brassicaceae	Isatis	tinctoria	Acetate 03	control 01	2		
Brassicaceae	Isatis	tinctoria	Acetate 03	control 01	2		
Brassicaceae	Nasturtium	officinale	Acetate 03	control 01	2		
Brassicaceae	Nasturtium	officinale	Acetate 03	control 01	2,0,0	1,0,0	
Buxaceae	Buxus	sinica	control 01	control 01			
Campanulaceae	Adenophora	bulleyana	Acetate 03	control 01	1,0,1		
Campanulaceae	Adenophora	bulleyana	Acetate 03	control 01	3		
Campanulaceae	Adenophora	bulleyana	Acetate 03	control 01	1,0,0		
Campanulaceae	Campanula	persicifolia	Acetate 03	control 01			
Campanulaceae	Codonopsis	pilosula	Acetate 03	control 01		2,0,3	
Campanulaceae	Codonopsis	pilosula	Acetate 03	control 01	1		1
Campanulaceae	Lobelia	siphilitica	Acetate 03	control 01			
Campanulaceae	Lobelia	siphilitica	Acetate 03	control 01	1,0,0		
Campanulaceae	Platycodon	grandiflorum	Acetate 03	control 01	1,0,0		
Campanulaceae	Platycodon	grandiflorum	Acetate 03	control 01			1,2
Caprifoliaceae	Sambucus	caerulea	Acetate 03	control 01	2,2	2,2	3,2
Caprifoliaceae	Sambucus	caerulea	Acetate 03	control 01	2,0,0		1,1
Caprifoliaceae	Sambucus	tigranli	Acetate 03	control 01			
Caprifoliaceae	Sambucus	tigranli	Acetate 03	control 01	2		
Caprifoliaceae	Symphoricarpos	albus	Acetate 03	control 01	3		
Caprifoliaceae	Symphoricarpos	albus	Acetate 03	control 01	3,0	2,0	1,1
Carvophyllaceae	Agrostemma	githago	Acetate 03	control 01	3,2	2,0	1,2
Carvophyllaceae	Agrostemma	githago	Acetate 03	control 01			
Carvophyllaceae	Gypsophila	paniculata	Acetate 03	control 01	1,0,0		
Carvophyllaceae	Gypsophila	paniculata	Acetate 03	control 01			
Carvophyllaceae	Herniaria	glabra	Acetate 03	control 01	2		
Carvophyllaceae	Herniaria	glabra	Acetate 03	control 01	1		
Carvophyllaceae	Lychnis	alba	Acetate 03	control 01	1		
Carvophyllaceae	Lychnis	alba	Acetate 03	control 01			

Caryophyllaceae	Lychnis	chalcadonica	control 01				3,3	
Caryophyllaceae	Lychnis	chalcadonica	Acetate 03				3,3	
Caryophyllaceae	Scleranthus	biflorus	control 01			2,0	2,0	
Caryophyllaceae	Scleranthus	biflorus	Acetate 03			2,0	2,0	
Caryophyllaceae	Silene	alba	control 01	2,1	2,0	2,2		2,2
Caryophyllaceae	Silene	alba	Acetate 03	2,3	2,2		3,4	2,2
Caryophyllaceae	Silene	amerita	control 01	1,0		3,0	2,2	2,2
Caryophyllaceae	Silene	amerita	Acetate 03	4,4	1,1	3,2	4,4	2,1
Celastraceae	Eunymus	koopmannii	control 01	1,1,1				
Celastraceae	Eunymus	koopmannii	Acetate 03					
Chenopodiaceae	Chenopodium	botrys	control 01					
Chenopodiaceae	Chenopodium	botrys	Acetate 03	2				
Cistaceae	Fumana	procumbens	control 01					
Cistaceae	Fumana	procumbens	Acetate 03					
Cistaceae	Fumana	procumbens	control 01					1,0
Cistaceae	Fumana	procumbens	control 01					2,0
Cistaceae	Fumana	procumbens	Acetate 03		1,0			
Cistaceae	Fumana	perforatum	control 01	3,0,2	1			
Cistaceae	Hypericum	perforatum	Acetate 03	3,0,1				
Colchicaceae	Gloriosa	superba	control 01					
Colchicaceae	Gloriosa	superba	Acetate 03			1	3	
Combretaceae	Terminalia	arjuna	control 01	4,3	2,2	3,2		2,2
Combretaceae	Terminalia	arjuna	Acetate 03	2,3	2,2	3,2		2,2
Commelinaceae	Commelina	coelestis	control 01	1,0			2,0	1,1
Commelinaceae	Commelina	coelestis	Acetate 03	2,2	2,1	2,2	3,2	1,1
Commelinaceae	Cyanditis	speciosa	control 01			5		
Commelinaceae	Cyanditis	speciosa	Acetate 03					
Commelinaceae	Tinaria	erecta	control 01	1,0	1,0	2,1	2,0	2,0
Commelinaceae	Tinaria	erecta	Acetate 03	4,4	1,1	5,3	2,0	1,1
Convolvulaceae	Mina	lobata	control 01			4		
Convolvulaceae	Mina	lobata	Acetate 03	4				
Cucurbitaceae	Cucumis	sativus	control 01					
Cucurbitaceae	Cucumis	sativus	control 01					
Cucurbitaceae	Cucumis	sativus	control 01					
Cucurbitaceae	Cucumis	sativus	Acetate 01					

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Cucurbitaceae	Cucumis	sativus	Acetate 01				
Cucurbitaceae	Cucumis	sativus	control 01				
Cucurbitaceae	Cucumis	sativus	Acetate 03	3,0,2	2,0,0		
Cucurbitaceae	Trichosanthes	angina	control 01				3,3
Cucurbitaceae	Trichosanthes	angina	Acetate 03				3,3
Cucurbitaceae	Thuja	orientalis	control 01	3,3,3			
Cupressaceae	Thuja	orientalis	Acetate 03	3,3,3			
Ebenaceae	Diospyros	kaki	control 01				
Ebenaceae	Diospyros	kaki	Acetate 03	3,0,0			
Elaeagnaceae	Elaeagnus	commutata	control 01				
Elaeagnaceae	Elaeagnus	commutata	Acetate 03	1,1,0			
Ericaceae	Rhododendron	luteum	control 01				
Ericaceae	Rhododendron	luteum	Acetate 03		2		
Euphorbiaceae	Pseudolachnostylis	maprouneifolia	control 01				2
Euphorbiaceae	Pseudolachnostylis	maprouneifolia	Acetate 03				
Euphorbiaceae	Albizia	julibrissin	control 01		3		
Fabaceae	Albizia	julibrissin	Acetate 03		3		
Fabaceae	Albizia	julibrissin	control 01				
Fabaceae	Albizia	julibrissin	Acetate 03				
Fabaceae	Albizia	julibrissin	control 01	1,0			
Fabaceae	Albizia	julibrissin	Acetate 03	2,2			
Fabaceae	Albizia	kolomikita	control 01	3			
Fabaceae	Albizia	kolomikita	Acetate 03				
Fabaceae	Amorpha	fruticosa	control 01	1,0		2,1	2,2
Fabaceae	Amorpha	fruticosa	Acetate 03	1,2	2,2	2,0	2,0
Fabaceae	Cassia	tora	control 01				1,1
Fabaceae	Cassia	tora	Acetate 03	3			
Fabaceae	Ceratonia	siliqua	control 01				
Fabaceae	Ceratonia	siliqua	Acetate 03	3			
Fabaceae	Ceratonia	siliqua	control 01				
Fabaceae	Ceratonia	siliqua	Acetate 03				
Fabaceae	Cicer	arietinum	control 01				
Fabaceae	Cicer	arietinum	Acetate 03	1,0,3			
Fabaceae	Clitoria	ternatea	control 01	2			5,4
Fabaceae	Clitoria	ternatea	Acetate 03	2			4
Fabaceae	Cytissus	scoparius	control 01	3,2,2			

Fabaceae	Cylissus	scoparius	Acetate 03	1,2,0			
Fabaceae	Dalea	candida	control 01	3,3,2			
Fabaceae	Dalea	candida	control up	3,3,2			
Fabaceae	Dalea	candida	Acetate 03	3,3,2			
Fabaceae	Erythrina	corallobendron	control 01				
Fabaceae	Erythrina	corallobendron	Acetate 03	2			
Fabaceae	Gleditsia	caspica	control 01				1,0
Fabaceae	Gleditsia	caspica	Acetate 03	2,3	1,1	2,1	
Fabaceae	Gleditsia	triacanthos	control 01				1,0
Fabaceae	Gleditsia	triacanthos	Acetate 03	1,2	3,0	2,1	2,0
Fabaceae	Gleditsia	triacanthos	Acetate 03		3,2		2,1
Fabaceae	Glycyrrhiza	echinata (glabra?)	control 01				
Fabaceae	Glycyrrhiza	echinata (glabra?)	Acetate 03	2			
Fabaceae	Gymnocladus	dioicus	control 01				
Fabaceae	Gymnocladus	dioicus	Acetate 03		2		
Fabaceae	Laburnum	anagyroides	control 01	4			
Fabaceae	Laburnum	anagyroides	Acetate 03	4			
Fabaceae	Laburnum	anagyroides	control 01				
Fabaceae	Laburnum	anagyroides	Acetate 03				
Fabaceae	Leucaena	leucocephala	control 01	2,2	3,0	2,0	
Fabaceae	Leucaena	leucocephala	Acetate 03	3,2			
Fabaceae	Lupinus	bicolor	control 01	2			
Fabaceae	Lupinus	bicolor	Acetate 03	2			
Fabaceae	Mimosa	pubica	control 01	4		2	
Fabaceae	Mimosa	pubica	Acetate 03	4		2	
Fabaceae	Ononis	spinosa	control 01				
Fabaceae	Ononis	spinosa	Acetate 03	2			
Fabaceae	Pachyrhizus	erosus	control 01				
Fabaceae	Pachyrhizus	erosus	Acetate 03	2,2,3			
Fabaceae	Parkinsonia	aculeata	control 01				
Fabaceae	Parkinsonia	aculeata	Acetate 03	3,2			
Fabaceae	Parkinsonia	aculeata	Acetate 03		2,0	2	
Fabaceae	Pelliphorum	pterocarpum	control 01				
Fabaceae	Pelliphorum	pterocarpum	Acetate 03				4
Fabaceae	Petalostemon	candidum	control 01	5			
Fabaceae	Petalostemon	candidum	Acetate 03	5			

Fabaceae	Pithecellobium	dulce	control 01		1,2		
Fabaceae	Pithecellobium	dulce	Acetate 03		1,1		3,0
Fabaceae	Prosopis	cineraria	control 01			3,1	
Fabaceae	Prosopis	cineraria	Acetate 03			2,1	
Fabaceae	Pueraria	lobata	control 01				
Fabaceae	Pueraria	lobata	Acetate 03	2	2		
Fabaceae	Schrankia	occidentalis	control 01				
Fabaceae	Schrankia	occidentalis	Acetate 03	1,3,1			
Fabaceae	Sophora	japonica	control 01	3			
Fabaceae	Sophora	japonica	Acetate 03	3			
Fabaceae	Spartium	juncum	control 01				
Fabaceae	Spartium	juncum	Acetate 03				2,3
Fabaceae	Spartium	juncum	control 01	3,0			
Fabaceae	Spartium	juncum	Acetate 03	3,0			
Fabaceae	Thermopsis	fabacea	control 01				
Fabaceae	Thermopsis	fabacea	Acetate 03				
Fabaceae	Thermopsis	fabacea	control 01				3
Fabaceae	Thermopsis	fabacea	Acetate 03	4			
Fabaceae	Thermopsis	montana	control 01				
Fabaceae	Thermopsis	montana	Acetate 03	3,0,0			
Fabaceae	Fagus	sylvatica	control 01	1,0			2,0
Fabaceae	Fagus	sylvatica	Acetate 03	1,2			
Fabaceae	Fagus	sylvatica	control 01	3			4,3
Flacourtiaceae	Dovyalis	calfra	Acetate 03	3			4,3
Flacourtiaceae	Dovyalis	calfra	control 01				
Gentianaceae	Centaureum	littorale	control 01				1
Gentianaceae	Centaureum	littorale	Acetate 03	1,0,0			1
Gentianaceae	Gentiana	macrophylla	control 01				1
Gentianaceae	Gentiana	macrophylla	Acetate 03				
Gentianaceae	Gentiana	tibetica	control 01				
Gentianaceae	Gentiana	tibetica	Acetate 03		4,0,0	3,0,2	
Geraniaceae	Erodium	ciutarium	control 01				
Geraniaceae	Erodium	ciutarium	Acetate 03	2,0,0	1,0,0		
Geraniaceae	Erodium	ciutarium	control 01				
Geraniaceae	Geranium	macrorrhizum	control 01				
Geraniaceae	Geranium	macrorrhizum	Acetate 03	4,2			
Geraniaceae	Geranium	macrorrhizum	control 01	3,0	3,0	1,0	2,0
Geraniaceae	Geranium	macrorrhizum	Acetate 03			5,5,5	
Hippocastanaceae	Aesculus	hippocastanum	control 01				

Hippocastanaceae	Aesculus	hippocastanum	Acetate 03			5,5,5	
Hippocastanaceae	Aesculus	woerflizensis	control 01			5,5,5	
Hippocastanaceae	Aesculus		Acetate 03			5,5,5	
Hyacinthaceae	Chionogalum	pomeridianum	control 01			5	
Hyacinthaceae	Chionogalum	pomeridianum	Acetate 03			5	
Hyacinthaceae	Hosta	fortunea	control 01			5,5,5	
Hyacinthaceae	Hosta	fortunea	Acetate 03			5,5,5	
Hydrophyllaceae	Nemophila	maculata	control 01				
Hydrophyllaceae	Nemophila	maculata	Acetate 03		2		
Hydrophyllaceae	Nemophila	maculata	control 01				
Hydrophyllaceae	Nemophila	maculata	Acetate 03				
Hydrophyllaceae	Phacelia	tanacetifolia	control 01				
Hydrophyllaceae	Phacelia	tanacetifolia	Acetate 03	2	1,0,0		
Hydrophyllaceae	Dierama	pulcherrimum	control 01				
Hydrophyllaceae	Dierama	pulcherrimum	Acetate 03			4,3	
Liliaceae	Leonurus	cardiaca	control 01				
Liliaceae	Leonurus	cardiaca	Acetate 03	4			2
Liliaceae	Monarda	citriodora	control 01				2
Liliaceae	Monarda	citriodora	Acetate 03				
Liliaceae	Monarda	citriodora	control 01	5			
Liliaceae	Monarda	citriodora	Acetate 03	4			
Liliaceae	Ocimum	sanctum	control 01				
Liliaceae	Ocimum	sanctum	Acetate 03				3,0
Liliaceae	Ocimum	sanctum	control 01				2,0
Liliaceae	Ocimum	sanctum	Acetate 03				
Liliaceae	Pogostemon	patchouli	control 01				
Liliaceae	Pogostemon	patchouli	Acetate 03	1			
Liliaceae	Salvia	dumetorum	control 01				
Liliaceae	Salvia	dumetorum	Acetate 03	3,4,4		2,0,3	
Liliaceae	Salvia	melifera	control 01		1,0,0		
Liliaceae	Salvia	melifera	Acetate 03		1,0,0		
Liliaceae	Schizonepeta	tenuifolia	control 01				
Liliaceae	Schizonepeta	tenuifolia	Acetate 03	3			
Liliaceae	Stachys	officinalis	control 01				
Liliaceae	Stachys	officinalis	Acetate 03	3			
Liliaceae	Cordylifera	australis	control 01		2,3		2,0

Liliaceae	Cordylone	australis	Acetate 03				
Liliaceae	Phormium	tenax	control 01	1,2	2,0	4,5	2,0
Liliaceae	Phormium	tenax	Acetate 03	2,2	2,0		
Linaceae	Linum	grandiflorum	control 01				
Linaceae	Linum	grandiflorum	Acetate 03				
Linaceae	Linum	grandiflorum	control 01				
Linaceae	Linum	grandiflorum	Acetate 03	2	2	2	
Linaceae	Linum	perenne	control 01	1			
Linaceae	Linum	perenne	Acetate 03	1			
Linaceae	Linum	usitatissimum	control 01				
Linaceae	Linum	usitatissimum	Acetate 03				
Linaceae	Linum	usitatissimum	control 01				
Loasaceae	Kissenia	capensis	Acetate 03	3			
Loasaceae	Kissenia	capensis	Acetate 03	2			
Loganiaceae	Buddleia	davidi	control 01				
Loganiaceae	Buddleia	davidi	Acetate 03	3			
Loganiaceae	Strychnos	spinosa	control 01				
Loganiaceae	Strychnos	spinosa	Acetate 03	4			
Magnoliaceae	Liriodendron	tulipifera	control 01				
Magnoliaceae	Liriodendron	tulipifera	Acetate 03	1			
Malvaceae	Abutilon	sp.	control 01				
Malvaceae	Abutilon	sp.	Acetate 03	5			
Malvaceae	Aithaea	officinalis	control 01				
Malvaceae	Aithaea	officinalis	Acetate 03				
Malvaceae	Aithaea	officinalis	control 01				
Malvaceae	Aithaea	officinalis	Acetate 03	2,0		0,0,3	
Malvaceae	Hibiscus	sabdariffa	control 01				
Malvaceae	Hibiscus	sabdariffa	Acetate 03		1,0,0	0,0,3	
Malvaceae	Lavatera	trimestris	control 01				
Malvaceae	Lavatera	trimestris	Acetate 03	5			
Malvaceae	Malva	mauritanica	control 01	3			
Malvaceae	Malva	mauritanica	Acetate 03	3			
Malvaceae	Malva	moschata	control 01				
Malvaceae	Malva	moschata	Acetate 03	5	2		
Melanthaceae	Melanthus	villosus	control 01				



Meliaceae	Melianthus	villosa	Acetate 03	4			
Molluginaceae	Pharnaceum	sp.	control 01				
Molluginaceae	Pharnaceum	sp.	Acetate 03		5		
Montiaceae	Montinia	caryophyllaceae	control 01				
Montiaceae	Montinia	caryophyllaceae	Acetate 03	2		3	
Moraceae	Maclura	pomifera	control 01	3,3,3			
Moraceae	Maclura	pomifera	Acetate 03	3,3,3			
Moraceae	Morus	alba	control 01	4			
Moraceae	Morus	alba	Acetate 03	4			
Moraceae	Morus	alba	control 01	5		3,0	
Moraceae	Morus	alba	Acetate 03	5		3,0	
Moraceae	Morus	nigra	control 01	2,0,2			
Moraceae	Morus	nigra	Acetate 03	2,0,2			
Nyctaginaceae	Abronia	nana	control 01	3,3,3	4,0,4		
Nyctaginaceae	Abronia	nana	Acetate 03	3,3,3			
Nyctaginaceae	Abronia	nana	Acetate 03	4,2,3	2,0,0		
Oleaceae	Ligustrum	lucidum	control 01	2,1	2,0		
Oleaceae	Ligustrum	lucidum	Acetate 03				
Oleaceae	Ligustrum	sinense	control 01				
Oleaceae	Ligustrum	sinense	Acetate 03				
Oleaceae	Ligustrum	sinense	control 01				
Oleaceae	Ligustrum	sinense	Acetate 03		3		
Oleaceae	Olea	europaea	control 01		2,0		
Oleaceae	Olea	europaea	Acetate 03	2,2	2,2		2,0
Onagraceae	Camissonia	cheiranthifolia	control 01				
Onagraceae	Camissonia	cheiranthifolia	Acetate 03				
Onagraceae	Camissonia	cheiranthifolia	control 01		3,0,0		
Onagraceae	Camissonia	cheiranthifolia	Acetate 03		3,0,0		
Onagraceae	Clarkia	amoena	control 01				
Onagraceae	Clarkia	amoena	Acetate 03		1,1,0	2,0,0	
Onagraceae	Clarkia	amoena	control 01				
Onagraceae	Clarkia	amoena	Acetate 03	3		5	
Onagraceae	Clarkia	unguiculata	control 01				
Onagraceae	Clarkia	unguiculata	Acetate 03	4		4	
Onagraceae	Oenothera	lamarckiana	control 01	2,0,0	1,0,0		
Onagraceae	Oenothera	lamarckiana	Acetate 03	2,0,0	1,0,0		

Papaveraceae	Chelidonium	maius	control 01	3.2.0		0.0.2		
Papaveraceae	Chelidonium	maius	Acetate 03	3.1.3		0.0.2		
Papaveraceae	Eschscholzia	californica	control 01					
Papaveraceae	Eschscholzia	californica	control 01					
Papaveraceae	Eschscholzia	californica	control 01					
Papaveraceae	Eschscholzia	californica	Acetate 02					
Papaveraceae	Eschscholzia	californica	Acetate 02					
Papaveraceae	Eschscholzia	californica	control 01	4		5	2.3	
Papaveraceae	Eschscholzia	californica	Acetate 03	4		5	2.3	
Papaveraceae	Eschscholzia	californica	control 01	4		3		
Papaveraceae	Eschscholzia	californica	Acetate 03	5	2	3		
Papaveraceae	Eschscholzia	californica	control 01	4				
Papaveraceae	Eschscholzia	californica	Acetate 03	5		5		
Papaveraceae	Eschscholzia	californica	control 01	5		5		
Papaveraceae	Eschscholzia	californica	Acetate 03	5		5	5.3	
Papaveraceae	Papaver	rheas	control 01					
Papaveraceae	Papaver	rheas	Acetate 03	4				
Papaveraceae	Dicorcarum	eriocarpum	control 01					
Pedaliaceae	Dicorcarum	eriocarpum	Acetate 03	5				
Pedaliaceae	Sesamum	indicum	control 01					
Pedaliaceae	Sesamum	indicum	Acetate 03	2				
Penaeaceae	Penaea	creatum	control 01	3				
Penaeaceae	Penaea	creatum	Acetate 03	3				
Pinaceae	Larix	olgensis	control 01	5		3		
Pinaceae	Larix	olgensis	Acetate 03	4		4.3		
Pinaceae	Pseudotsuga	glauca	control 01	1.1		1.0		
Pinaceae	Pseudotsuga	glauca	Acetate 03	2.3				2.0
Pittosporaceae	Pittosporum	tobira	control 01	1		1.0.0		
Pittosporaceae	Pittosporum	tobira	Acetate 03	1		1.0.5		
Pittosporaceae	Pittosporum	viridiflorum	control 01					
Pittosporaceae	Pittosporum	viridiflorum	Acetate 03					
Pittosporaceae	Pittosporum	viridiflorum	control 01	4		5		
Pittosporaceae	Pittosporum	viridiflorum	Acetate 03	4		5		
Pittosporaceae	Sollya	heterophylla	control 01	4.4			2.0	
Pittosporaceae	Sollya	heterophylla	Acetate 03	2.3c	1.2	3.2	3.3	3.0



Restionaceae	Calopsis	paniculata	control 01					
Restionaceae	Calopsis	paniculata	control 01			2		
Restionaceae	Calopsis	paniculata	Acetate 03			2		
Rhamnaceae	Ceanothus	cuneatus	control 01	0,0,3	4,0,0			
Rhamnaceae	Ceanothus	cuneatus	Acetate 03	5,0,4				
Rhamnaceae	Ceanothus	integerrimus	control 01	5	5			
Rhamnaceae	Ceanothus	integerrimus	Acetate 03	5+				
Rhamnaceae	Hovenia	dulcis	control 01	4,4			2,2	1,2
Rhamnaceae	Hovenia	dulcis	Acetate 03	3,3		2,0		2,0
Rhamnaceae	Pallurus	spina-christi	control 01					
Rhamnaceae	Pallurus	spina-christi	Acetate 03	1,1,0				2
Rhamnaceae	Rhamnus	ilicifolia	control 01					
Rhamnaceae	Rhamnus	ilicifolia	Acetate 03	2				
Rhamnaceae	Rhamnus	juluba	control 01	4				
Rhamnaceae	Rhamnus	juluba	Acetate 03	4				
Rhamnaceae	Rhamnus	spina-christi	control 01	4				
Rhamnaceae	Rhamnus	spina-christi	Acetate 03	4	5			
Rosaceae	Agrimonia	eupatoria	control 01					
Rosaceae	Agrimonia	eupatoria	Acetate 03	2,0,0				
Rosaceae	Amelanchier	ovalis	control 01				2,1	
Rosaceae	Amelanchier	ovalis	Acetate 03			2,0		
Rosaceae	Amelanchier	utahensis	control 01					
Rosaceae	Amelanchier	utahensis	Acetate 03	2,1	3,2	2,2	1,1	1,0
Rosaceae	Anuncus	dioicus	control 01	2,0,0	2,1,2	2,0,0		
Rosaceae	Anuncus	dioicus	Acetate 03	2,0,0	2,1,2	2,0,0		
Rosaceae	Aruncus	arnoldiana	control 01	2,2				3,0
Rosaceae	Crataegus	arnoldiana	Acetate 03		1,0	3,0		2,0
Rosaceae	Crataegus	coccinoides	control 01			2,0		1,0
Rosaceae	Crataegus	coccinoides	Acetate 03			2,0		1,1
Rosaceae	Geum	faurieri	control 01	5,0,0				
Rosaceae	Geum	faurieri	Acetate 03	3,0,0	4,0,0			
Rosaceae	Holodiscus	discolor	control 01					
Rosaceae	Holodiscus	discolor	Acetate 03					
Rosaceae	Holodiscus	discolor	control 01					
Rosaceae	Holodiscus	discolor	Acetate 03	2,2	1,2			2,3
Rosaceae	Prinsepia	sibirica	control 01					

Rosaceae	Prinsepia	sinensis	Acetate 03				
Rosaceae	Prinsepia	sinensis	control 01	3			
Rosaceae	Prinsepia	sinensis	Acetate 03	3			
Rosaceae	Prinsepia	uniflora	control 01		1,0		
Rosaceae	Prinsepia	uniflora	Acetate 03		1,0		1,0
Rosaceae	Prunus	lyonii	control 01		2,0	2,0	
Rosaceae	Prunus	lyonii	Acetate 03		1,0	3,2	
Rosaceae	Pyrus	communis	control 01		1,1		
Rosaceae	Pyrus	communis	Acetate 03		1,2		1,0
Rosaceae	Pyrus	communis	Acetate 03	1,2			
Rosaceae	Rosa	canina	control 01				2,0
Rosaceae	Rosa	canina	Acetate 03				2,0
Rosaceae	Rosa	roxburghii	control 01	2c,2			2,2
Rosaceae	Rosa	roxburghii	Acetate 03	2c,2			2,3
Rutaceae	Ptelea	trifoliata	control 01				2,2
Rutaceae	Ptelea	trifoliata	Acetate 03	4			
Rutaceae	Ruta	graveolens	control 01	5		3	
Rutaceae	Ruta	graveolens	Acetate 03	5		3	
Sapindaceae	Sapindus	mukorossi	control 01				
Sapindaceae	Sapindus	mukorossi	Acetate 03		1,1		
Sapindaceae	Xanthoxerus	sorbifolius	control 01	3		5	
Sapindaceae	Xanthoxerus	sorbifolius	Acetate 03	3		5	
Sapotaceae	Sideroxylon	linerne	control 01				
Sapotaceae	Sideroxylon	linerne	Acetate 03		2		
Saxifragaceae	Asilbe	chinensis	control 01				
Saxifragaceae	Asilbe	chinensis	Acetate 03	2		2	
Saxifragaceae	Heuchera	sanguinea	control 01	3,2	1,0	3,3	
Saxifragaceae	Heuchera	sanguinea	Acetate 03	3,2	1,2	4,4	2,2
Saxifragaceae	Heuchera	sanguinea	control 01	3,2	2,0		
Saxifragaceae	Heuchera	sanguinea	Acetate 03	3,2	2,2		2,2
Scrophulariaceae	Digitalis	grandiflora	control 01				
Scrophulariaceae	Digitalis	grandiflora	Acetate 03			2,0,0	
Scrophulariaceae	Linaria	maroccana	control 01				
Scrophulariaceae	Linaria	maroccana	Acetate 03	2			
Scrophulariaceae	Linaria	vulgaris	control 01				
Scrophulariaceae	Linaria	vulgaris	Acetate 03			1,0,1	

Scrophulariaceae	Paulownia	tomentosa	control 01					
Scrophulariaceae	Paulownia	tomentosa	Acetate 03	2				
Scrophulariaceae	Veronica	officinalis	control 01	2				
Scrophulariaceae	Veronica	officinalis	Acetate 03	2				
Solanaceae	Datura	stramonium	control 01					
Solanaceae	Datura	stramonium	control 01					
Solanaceae	Datura	stramonium	Acetate 01					
Solanaceae	Datura	stramonium	Acetate 01					
Solanaceae	Datura	stramonium	Acetate 01					
Solanaceae	Datura	stramonium	control 01					
Solanaceae	Datura	stramonium	Acetate 03	1,0,0				
Solanaceae	Hyoscyamus	albus	control 01	2,2	2,1	2,2		3,2
Solanaceae	Hyoscyamus	albus	Acetate 03	3,3	2,0	2,2		2,2
Solanaceae	Nicandra	physaloides	control 01	1,0,1	1,0,0			
Solanaceae	Nicandra	physaloides	Acetate 03	1,0,1	1,0,1			
Solanaceae	Petunia	hybrida	control 01	2,2				
Solanaceae	Petunia	hybrida	Acetate 03	2,2			2,2	2,3
Syracaceae	Syrax	obassia	control 01			5		
Syracaceae	Syrax	obassia	Acetate 03	1	5			
Theaceae	Camellia	japonica	control 01			5,5,5		
Theaceae	Camellia	japonica	Acetate 03			5,5,5		
Tropaeolaceae	Tropaeolum	peregrinum	control 01			5		
Tropaeolaceae	Tropaeolum	peregrinum	Acetate 03					
Ulmaceae	Ulmus	parvifolia	control 01	2,0		1,3c		2c,4
Ulmaceae	Ulmus	parvifolia	Acetate 03	2,2		1,2		2c,2
Valerianaceae	Valeriana	officinalis	control 01					
Valerianaceae	Valeriana	officinalis	Acetate 03	3,0,2				
Verbenaceae	Caryopteris	sp.	control 01	3,1,3				
Verbenaceae	Caryopteris	sp.	Acetate 03	3,1,3				
Verbenaceae	Gmelina	arbores	control 01		3,2			
Verbenaceae	Gmelina	arbores	Acetate 03	2,2	3,1			
Violaceae	Viola	odorata	control 01					3,2
Violaceae	Viola	odorata	Acetate 03	2,2	1,0	2,0		
Zingiberaceae	Elettaria	cardamomum	control 01	1,0,0	1,0,0	3,0,0		
Zingiberaceae	Elettaria	cardamomum	Acetate 03	1,0,0	1,1,1			

Zygophyllaceae	Tribulus	terrestris	control 01				
Zygophyllaceae	Tribulus	terrestris	Acetate 03				
Zygophyllaceae	Tribulus	terrestris	control 01			1,00	
Zygophyllaceae	Tribulus	terrestris	Acetate 03				

The anti-microbial data collected in Table 6 is presented in terms of a scale from 0 (no growth inhibition) to 5 (essentially complete growth inhibition). Results from independent trials of the same extract exposed to the same microbe are separated by commas. The data indicate that a variety of plants exhibit reproducible anti-microbial activity (anti-bacterial, anti-fungal, or both activities) following elicitation of the plants with acetic acid.

Of the many plant extracts subjected to the anti-microbial assay, the following plants yielded extracts having no detectable anti-microbial activity, whether induced by acetic acid or not: *Baphicacanthus cusia*, *Actinidia chinensis*, *Beaucarnea recurvata*, *Aloinopsis luckhoffii*, *Aloinopsis rubrolineata*, *Delosperma ashtonii*, *Ruschia indurata*, *Ruschia pulvinaris*, *Tetragonia decumbens*, *Allium cepa*, *Allium schoenoprasum*, *Allium tuberosum*, *Achyranthes bidentata*, *Amaranthus tricolor*, *Celosia cristata*, *Cyathula officinalis*, *Pistacia chinensis*, *Pistacia vera*, *Rhus ovata*, *Annona cherimola*, *Annona senegalensis*, *Asimina triloba*, *Anethum graveolens*, *Angelica polymorpha*, *Apium graveolens*, *Carum carvi*, *Carum copticum*, *Coriandrum sativum*, *Daucus carota*, *Heracleum sphondylium*, *Pimpinella anisum*, *Saposhnikovia divaricata*, *Carissa grandiflora*, *Rauwolfia caffra*, *Acorus calamus*, *Monstera deliciosa*, *Zantedeschia aethiopica*, *Zantedeschia albomaculata*, *Aralia racemosa*, *Fatsia japonica*, *Aristolochia clematis*, *Asclepias curassavica*, *Asclepias tuberosa*, *Protasparagus africanus*, *Gasteria acinacifolia*, *Trachyandra divaricata*, *Trachyandra revoluta*, *Achillea ptarmica*, *Ageratum conyzoides*, *Antennaria dioica*, *Arctium lappa*, *Arnica chamissonis*, *Artemisia annua*, *Artemisia apiacea*, *Aster chilensis*, *Aster novae-angliae*, *Athrix elata*, *Bellis perennis*, *Calendula officinalis*, *Carlina acaulis*, *Cichorium endivia*, *Cosmos sulphureus*, *Cynara cardunculus*, *Cynara scolymus*, *Echinacea angustifolia*, *Echinacea pallida*, *Echinacea purpurea*, *Eclipta alba*, *Erigeron glaucus*, *Eriophyllum confertiflorum*, *Grindelia robusta*, *Helenium autumnale*, *Helianthus annuus*, *Hieracium pilosella*, *Inula helenium*, *Lactuca sativa*, *Layia platyglossa*, *Leontopodium alpinum*, *Rudbeckia hirta*, *Schoenia cassiniana*, *Serratula tinctoria*, *Silphium laciniatum*, *Spilanthes oleracea*, *Stokesia laevis*, *Tagetes minuta*, *Taraxacum officinale*, *Telekia speciosa*, *Trichostemma lanatum*, *Tussilago farfara*, *Xanthium sibiricum*, *Impatiens balsamina*, *Begonia sutherlandii*, *Berberis julianae*, *Berberis poretii*, *Alnus japonica*, *Betula nigra*, *Betula pendula*, *Carpinus betulus*, *Corylus avellana*, *Jacaranda mimosifolia*, *Adansonia digitata*, *Borago officinalis*, *Cynoglossum firmament*, *Echium vulgare*, *Heliotropium arborescens*, *Lithospermum*



officinale, *Lythrum officinalis*, *Barbarea verna*, *Blossa campestris*, *Blossa nigra*, *Cochlearia officinalis*, *Crambe aspera*, *Crambe tatarica*, *Hesperis matronalis*, *Iberis amara*, *Iberis intermedia*, *Isatis indigotica*, *Lepidium sativum*, *Lunaria annua*, *Raphanus sativus*, *Berzelia abrotanoides*, *Brunia albiflora*, *Brunia nodiflora*, *Opuntia humifusa*, *Calycanthus floridus*, *Chimonanthus praecox*, *Campanula rapunculus*, *Campanula trachelium*, *Platycodon grandiflorus*, *Wahlenbergia undulata*, *Cannabis sativa*, *Capparis inermis*, *Cleome spinosa*, *Kolkwitzia amabilis*, *Lonicera chamissoi*, *Lonicera maackii*, *Lonicera tatarica*, *Sambucus mexicana*, *Sambucus nigra*, *Viburnum prunifolium*, *Viburnum trilobum*, *Weigela florida*, *Carica papaya*, *Cerastium tomentosum*, *Dianthus andrzejkowskianus*, *Dianthus barbatus*, *Dianthus gratianopolitanus*, *Lychnis coriacea*, *Sagina subulata*, *Saponaria officinalis*, *Stellaria media*, *Casuarina cunninghamiana*, *Casuarina equisetifolia*, *Maytenus capitata*, *Atriplex hortensis*, *Beta vulgaris*, *Chenopodium ambrosioides*, *Chenopodium nuttalliae*, *Cistus incanus*, *Cistus ladanifer*, *Helianthemum grandiflorum*, *Hypericum montanum*, *Hypericum olympicum*, *Androcymbium pulchrum*, *Gloriosa carsonii*, *Gloriosa rothschildiana*, *Terminalia brachystemma*, *Terminalia mantaly*, *Commelina communis*, *Polia japonica*, *Tradescantia virginiana*, *Convolvulus cantabrica*, *Ipomoea purpurea*, *Ipomoea tricolor*, *Pharbitis nil*, *Camptotheca acuminata*, *Nyssa aquatica*, *Sedum pulchellum*, *Sedum telephium*, *Bryonia alba*, *Citrullus colorynthi*, *Cucumis melo*, *Cucumis metuliferus*, *Cucurbita pepo*, *Lagenaria siceraria*, *Luffa aegyptiaca*, *Momordica charantia*, *Trichosanthes kirilowii*, *Cunonia capensis*, *Calocedrus decurrens*, *Cupressus lusitanica*, *Cupressus sempervirens*, *Thuja occidentalis*, *Cladium mariscus*, *Cyperus esculentus*, *Scirpus californicus*, *Scirpus robustus*, *Dioscorea batatas*, *Dioscorea dregeana*, *Dipsacus fullonum*, *Dipsacus sativus*, *Knautia arvensis*, *Scabiosa caucasica*, *Scabiosa lucida*, *Succisa pratensis*, *Dracaena hookeriana*, *Diospyros virginiana*, *Elaeagnus angustifolia*, *Ephedra nevadensis*, *Ephedra viridis*, *Croton californicus*, *Euphorbia lathyris*, *Ricinus communis*, *Securinega suffruticosa*, *Euptelea pleiosperma*, *Abrus precatorius*, *Acacia saligna*, *Albizia lebeck*, *Arachis hypogaea*, *Astragalus membranaceus*, *Astragalus sinicus*, *Baptisia australis*, *Cajanus cajan*, *Cassia abbreviata*, *Chamaecrista fasciculata*, *Colutea arborescens*, *Cytissus albus*, *Dolichos biflorus*, *Erythrina christa-galli*, *Erythrina flabelliformis*, *Galega officinalis*, *Genista tinctoria*, *Glycine max*, *Glycyrrhiza echinata*, *Glycyrrhiza glabra*, *Glycyrrhiza glabra (echinata?)*, *Indigofera suffruticosa*, *Indigofera tinctoria*, *Lablab purpureus*, *Lens culinaris?*, *Lespedeza capitata*,

Lupinus arboreus, Lupinus luteus, Lupinus nanus, Lupinus perennis, Lupinus polyphyllus, Lupinus propinquus, Medicago sativa, Petalostemon purpureum, Phaseolus mungo, Prosopis glandulosa, Psoralea pinnata, Pterocarpus indicum, Pueraria thunbergiana, Robinia pseudoacacia, Tamarindus indica, Tephrosia grandiflora, Tephrosia vogelii, Trifolium pratense, Trifolium sp., Trigonella foenum-graecum, Vigna unguiculata, Wisteria sinensis, Castanea dentata, Scolopia zeyheri, Centaurium erythraea, Gentiana lutea, Geranium maculatum, Geranium sibiricum, Ginkgo biloba, Globularia aphyllanthes, Globularia trichosanthes, Albuca altissima, Lachenalia pusilla, Philadelphus incanus, Nemophila menziesii, Phacelia campanularia, Belamcanda chinensis, Gladiolus cunonius, Iris germanica, Iris notha, Iris pseudoacorus, Iris sibirica, Carya aquatica, Juglans nigra, Juglans regia, Juncus acutus, Acinos thymoides, Agastache foeniculum, Agastache mexicana, Agastache nepetoides, Agastache rugosa, Ballota nigra, Calamintha nepeta, Clinopodium vulgare, Dracocephalum moldavica, Dracocephalum scrobiculatum, Elsholtzia stauntonii, Glechoma hederacea, Hyssopus officinalis, Lavandula officinalis, Leonotis nepetifolia, Leonurus sibiricus, Lycopus europaeus, Lycopus europeus, Marrubium incanum, Marrubium vulgare, Mentha spicata, Monarda didyma, Monarda fistulosa, Nepeta cataria, Ocimum basilicum, Ocimum gratissimum, Origanum hirtum, Perilla frutescens, Prunella vulgaris, Pycnanthemum pycnanthemoides, Rosmarinus officinalis, Salvia apiana, Salvia columbariae, Salvia leucophylla, Salvia officinalis, Salvia sclarea, Satureja hortensis, Scutellaria altissima, Scutellaria cretica, Scutellaria orientalis, Sideritis syriaca, Stachys byzantina, Teucrium chamaedrys, Teucrium scorodonia, Thymus cretaceus, Thymus vulgaris, Trichostema lanatum, Lanaria lanata, Stauntonia hexaphylla, Cinnamomum camphora, Laurus nobilis, Lilium martagon, Mentzelia lindleyi, Galpinia transvaalica, Lagerstroemia indica, Lawsonia inermis, Magnolia grandiflora, Acridocarpus natalitius, Alcea rosea, Althaea broussonetifolia, Althaea cannabina, Gossypium herbaceum, Hibiscus trionum, Malacothamnus fasciculatus, Dissotis senegambiensis, Pseudosbeckia swynertonii, Entandrophragma caudatum, Toona serrata, Melianthus major, Ficus religiosa, Morina longifolia, Myrica pensylvanica, Callistemon citrinus, Melaleuca alternifolia, Myrthus communis, Psidium guajava, Abronia villosa, Mirabilis jalapa, Forsythia suspensa, Fraxinus excelsior, Fraxinus pennsylvanica, Maytenus capitata, Syringa villosa, Camissonia pallida, Clarkia rubicunda, Oenothera biennis, Oenothera speciosa, Paeonia suffruticosa, Argemone mexicana, Eschscholzia

*Cucurbita*, *Papaver dubium*, *Papaver orientale*, *Passiflora caerulea*, *Passiflora edulis*, *Ceratotheca sesamoides*, *Harpagophytum procumbens*, *Harpagophytum zeyheri*, *Phytolacca americana*, *Abies balsamea*, *Abies nephrolepis*, *Cedrus atlantica*, *Cedrus deodara*, *Pinus pinea*, *Plantago asiatica*, *Plantago erecta*, *Plantago hirtella*, *Plantago sempervirens*, *Plantago squallida*, *Platanus occidentalis*, *Armeria juniperifolia*, *Armeria maritima*, *Limonium gmeinii*, *Bouteloua curtipendula*, *Cortaderia selloana*, *Cymbopogon flexuosus*, *Festuca heterophylla*, *Festuca rubra*, *Merxmuellera arundinacea*, *Panicum virgatum*, *Polypogon monspeliensis*, *Schizachyrium scoparium*, *Setaria sphacelata*, *Sorghum sudanense*, *Vetiveria zizanioides*, *Podocarpus falcatus*, *Eriastrum densifolium*, *Phlox drummondii*, *Phlox paniculata*, *Securidaca longepedunculata*, *Eriogonum latifolium*, *Eriogonum umbellum*, *Polygonum hydropiper*, *Polygonum lapathifolium*, *Polygonum odoratum*, *Polygonum orientale*, *Polygonum punctatum*, *Rheum altaicum*, *Rheum palmatum*, *Rumex acetosa*, *Rumex confertus*, *Rumex crispus*, *Rumex euxinus*, *Rumex obtusifolius*, *Calandrinia ciliata*, *Claytonia perfoliata*, *Portulacca oleracea*, *Agallia arvensis*, *Lysimachia punctata*, *Primula elatior*, *Aconitum napellus*, *Adonis aestivalis*, *Anemone pulsatilla*, *Aquilegia formosa*, *Aquilegia transsilvanica*, *Aquilegia vulgaris*, *Clematis manschurica*, *Clematis virginiana*, *Consolida orientalis*, *Nigella arvensis*, *Nigella damascena*, *Nigella sativa*, *Thalictrum flavum*, *Trollius europaeus*, *Reseda luteola*, *Reseda odorata*, *Rhamnus cathartica*, *Agrimonia pilosa*, *Aruncus kamtschaticus*, *Cercocarpus betuloides*, *Chaenomeles japonica*, *Filipendula vulgaris*, *Geum urbanum*, *Pentaphylloides mandschurica*, *Physocarpus opulifolius*, *Potentilla recta*, *Potentilla tormentilla*, *Poterium sanguisorba*, *Pyracantha coccinea*, *Rosa damascena*, *Sanguisorba officinalis*, *Sorbaria arborea*, *Sorbaria sorbifolia*, *Cephalanthus occidentalis*, *Galium aparine*, *Galium spurium*, *Galium verum*, *Gardenia jasminoides*, *Rubia tinctorum*, *Evodia daniellii*, *Evodia hupehensis*, *Phellodendron amurense*, *Cardiospermum grandiflorum*, *Cardiospermum halicacabum*, *Koelreuteria paniculata*, *Litchi chinensis*, *Anemopsis californica*, *Bergenia cordifolia*, *Bergenia crassifolia*, *Heuchera pilosissima*, *Antirrhinum majus-maximum*, *Castilleja exserta*, *Collinsia canadensis*, *Collinsia heterophylla*, *Digitalis lanata*, *Digitalis purpurea*, *Gratiola officinalis*, *Hebenstreitia fastigiosa*, *Mimulus guttatus*, *Mimulus puniceus*, *Penstemon barbatus*, *Scrophularia nodosa*, *Selago corymbosa*, *Verbascum thapsus*, *Veronica spicata*, *Ailanthus altissima*, *Kirkia acuminata*, *Simmondsia chinensis*, *Atropa bella-donna*, *Brugmansia suaveolens*, *Capsicum annuum*, *Capsicum chinense*, *Datura innoxia*, *Lycium*

barbatum, Lycopersicon esculentum, Physalis ixocarpa, Solanum aculeatissimum, Solanum dulcamara, Solanum melongena, Withania somnifera, Brachychiton discolor, Sterculia africana, Sterculia quinqueloba, Strelitzia nicolae, Strelitzia reginae, Cunninghamia lanceolata, Taxodium distichum, Grewia biloba, Grewia flavescens, Grewia pachycalyx, Tropaeolum majus, Typha latifolia, Celtis occidentalis, Ulmus americana, Ulmus rubra, Zelkova sinica, Urtica dioica, Valerianella locusta, Verbena hastata, Verbena officinalis, Verbena tenuisecta, Vitex agnus-castus, Cyphostemma juttiae, Vitis amurensis, Vitis vinifera, Zingiber officinale, Balanites maughamii, Larrea tridentata, and Peganum harmala.

Additionally, the following plants responded to acetic acid by yielding extracts that inhibited an anti-microbial activity observed in the extracts of un-elicited plants: *Ammi majus*, *Eryngium campestre*, *Hydrocotyle asiatica*, *Levisticum officinale*, *Phoenix reclinata*, *Bulbine aloides*, *Machaeranthera bigelovii*, *Parthenium integrifolium*, *Ratibida columnifera*, *Iberis pinnata*, *Trichocereus pachanoi*, *Campanula carpatica*, *Humulus lupulus*, *Hypericum androsaemum*, *Sedum spurium*, *Cupressus sargentii*, *Hippophaë rhamnoides*, *Ephedra nevadensis*, *Ephedra viridis*, *Oxydendrum arboreum*, *Eremocarpus setigerus*, *Acacia redolens*, *Cassia hebecarpa*, *Delonix regia*, *Lupinus densiflorus*, *Lupinus luteus*, *Lupinus nanus*, *Lupinus perennis*, *Lupinus polyphyllus*, *Lupinus propinquus*, *Lupinus succulentus*, *Mucuna deeringiana*, *Robinia fertilis*, *Phacelia bolanderi*, *Iris versicolor*, *Scutellaria baicalensis*, *Limnanthes douglasii*, *Convallaria majalis*, *Linum hirsutum*, *Alcea nigra*, *Toona sinensis*, *Melianthus comosus*, *Morus rubra*, *Morina longifolia*, *Myrica cerifera*, *Myrica pennsylvanica*, *Callistemon citrinus*, *Leptospermum scoparium*, *Syringa amurensis*, *Syringa pekinensis*, *Syringa villosa*, *Syringa vulgaris*, *Gymnaglossum officinale*, *Papaver bracteatum*, *Proboscidea louisianica*, *Abies firma*, *Bauheia*, *Primula japonica*, *Grevillea robusta*, *Punica granatum*, *Paliurus hemsleyanus*, *Cydonia oblonga*, *Malus communis*, *Tellima grandiflora*, *Schisandra chinensis*, *Antirrhinum majus-maximum*, *Castilleja exserta*, *Chelone glabra*, *Vernonia noveboracensis*, *Hyoscyamus niger*, *Firmiana simplex*, *Fremontodendron californicum*, *Cryptomeria japonica*, *Camellia sinensis*, *Vitex negundo*, *Vitis labrusca*, *Vitis vinifera*, *Welwitschia mirabilis*, and *Cnidium monnieri*.

While the data described above indicate that some plants do not contain detectable levels of an anti-microbial activity, and others show activity both with and without elicitation, it is significant that approximately 40% of all active plant species exhibited activity only after

elicitation with acetic acid. Without wishing to be bound by theory, the data suggest that new biosynthetic pathways are being triggered, resulting in the accumulation of novel biologically active substances and/or activation of production pathways for existing biologically active substances is occurring, resulting in pronounced antimicrobial activities, and therefore in potential new drug development. Using the growth regimens described herein, e.g., hydroponic plant growth under controlled nutrient and light conditions, the results have been shown to be highly reproducible (approximately 80% of all trials with a given plant yield consistent outcomes in terms of anti-microbial activity), in contrast to the variations typically seen when investigating plant material collected following traditional protocols in drug discovery programs from natural sources.

One of ordinary skill in the art will recognize that many variations of the above-described bioassay exist and are suitable for testing extracts of plants elicited with acetic acid for anti-microbial activity. For example, anti-bacterial bioassays may be conducted by delivering extracts to MDS Pharma Services, Taipei, Taiwan, for testing in its standard anti-microbial screening assay.

#### **Example 9: Anti-cancer Activity Elicited by Acetic Acid**

The anti-cancer activity of extracts from control and elicited plants was assessed through collaboration with the National Cancer Institute (NCI). Extracts of plant roots were generally prepared according to the protocol described in Example 4. In particular, plant roots (approximately 1 - 5 g fresh weight) were harvested and freeze-dried, followed by an extraction with 20 ml of 80% (v/v) methanol per gram of lyophilized roots at room temperature for 48 hours. The methanol-root compositions are then centrifuged and the supernatant is decanted. The extract is formed by drying the supernatant by evaporation. Typically, 1 - 2 mg of extract were used in each bioassay.

The anti-cancer assays were conducted using a panel of three cancer cell lines, breast (NCI line MCF-7), melanoma (NCI line UACC-62), and renal (NCI line TK-10) cancer cell lines, or breast (NCI line MCF-7), central nervous system (NCI line SF-268), and non-small cell lung (NCI line NCI-H460) cancer cell lines. **[Do we have to worry about biological deposits and ATCC information here?]** A single-dose, 48-hour continuous exposure protocol was used and a sulforhodamine B (SRB) protein assay was used to estimate cancer

cell growth. Anti-cancer activities have been expressed as percent growth inhibition. The numbers in the cells representing the detected anti-cancer activity are percentage growth of the corresponding cancer cells, calculated according to one of the following equations:

$$\frac{100 \times (\text{MeanODtest} - \text{MeanODtzero})}{(\text{MeanODctrl} - \text{MeanODtzero})} \quad \text{If } (\text{MeanODtest} - \text{MeanODtzero}) \geq 0, \text{ then } PG =,$$

$$\frac{100 \times (\text{MeanODtest} - \text{MeanODtzero})}{(\text{MeanODtzero})} \quad \text{or if } (\text{MeanODtest} - \text{MeanODtzero}) < 0, \text{ then } PG =,$$

If  $(\text{MeanODtest} - \text{MeanODtzero}) > 0$ , then  $PG =$ ,  
 or if  $(\text{MeanODtest} - \text{MeanODtzero}) < 0$ , then  $PG =$ ,  
 where:

$PG$  is percent growth;

$\text{MeanODtzero}$  is the average of two optical density (OD) measurements of SRB-derived color in a cell culture just before exposure of the cancer cells to the plant extract;

$\text{MeanODtest}$  is the average of two OD measurements of SRB-derived color in a cell culture after 48 hours exposure of the cancer cells to the plant extract; and

$\text{MeanODctrl}$  is the average of two OD measurements of SRB-derived color in a cell culture after 48 hours with no exposure of cancer cells to plant extract.

The plant species screened for anti-cancer activity are identified in Table 7, below.

**Table 7. Anti-cancer activity of extracts elicited with acetic acid**

Family	Genus	species	treatment	Breast	Melanoma	Renal	CNS	Lung
Agavaceae	Agave	chrysantha	control 01					
Agavaceae	Agave	chrysantha	Acetate 03	-37			-14	-49
Anacardiaceae	Rhus	integrifolia	control 01				-15	
Anacardiaceae	Rhus	integrifolia	Acetate 03	-13				
Anacardiaceae	Rhus	ovata	control 01					
Anacardiaceae	Rhus	ovata	Acetate 03	-24			-25	-20
Anacardiaceae	Rhus	typhina	control 01					
Anacardiaceae	Rhus	typhina	Acetate 03	-62			-68	-47
Asteraceae	Anthemis	nobilis	control 01					-71
Asteraceae	Anthemis	nobilis	Acetate 03	-47				
Asteraceae	Bellis	perennis	control 01				-84	-89
Asteraceae	Bellis	perennis	Acetate 03	-79				
Asteraceae	Calendula	officinalis	control 01				-51	
Asteraceae	Calendula	officinalis	Acetate 03	-38				
Asteraceae	Chrysanthemum	coronarium	control 01	-25				
Asteraceae	Chrysanthemum	coronarium	Acetate 03				-69	-60
Asteraceae	Chrysanthemum	coronarium	control 01					
Asteraceae	Chrysanthemum	parthenium	control 01				-37	
Asteraceae	Chrysanthemum	parthenium	Acetate 03					
Asteraceae	Lasthenia	glabrata	control 01				-6	
Asteraceae	Lasthenia	glabrata	Acetate 03					
Asteraceae	Lavla	platyglossa	control 01	-25			-79	
Asteraceae	Lavla	platyglossa	Acetate 03					
Asteraceae	Solidago	virgaurea	control 01				-62	
Asteraceae	Solidago	virgaurea	Acetate 03					
Asteraceae	Solidago	virgaurea	control 01					
Berberidaceae	Berberis	thunbergii	control 01	-50	-57			
Berberidaceae	Berberis	thunbergii	Acetate 03	-5	-31			
Berberidaceae	Berberis	thunbergii	control 01					
Berberidaceae	Berberis	thunbergii	Acetate 03					
Betulaceae	Alnus	japonica	control 01					
Betulaceae	Alnus	japonica	Acetate 03	-49	-22			

Betulaceae	Alnus	japonica	control 01						
Brassicaceae	Barbarea	verna	control 01						
Brassicaceae	Barbarea	verna	Acetate 03						-17
Brassicaceae	Iberis	intermedia	control 01					-25	
Brassicaceae	Iberis	intermedia	Acetate 03					-12	-60
Burseraceae	Bursera	modiflora	Acetate 03	-6				-2	-24
Caryophyllaceae	Dianthus	andzelewskia	control 01				-93	-97	
Caryophyllaceae	Dianthus	andzelewskia	Acetate 03				-65	-79	
Caryophyllaceae	Gypsophila	paniculata	control 01						
Caryophyllaceae	Gypsophila	paniculata	Acetate 03						-85
Caryophyllaceae	Lychnis	chalcidonica	control 01						
Caryophyllaceae	Lychnis	chalcidonica	Acetate 03						
Chenopodiaceae	Atriplex	hortensis	control 01						-79
Chenopodiaceae	Atriplex	hortensis	Acetate 03				-67		
Chenopodiaceae	Atriplex	hortensis	control 01						
Chenopodiaceae	Atriplex	hortensis	Acetate 03						
Cucurbitaceae	Bryonia	alba	control 01					-2	
Cucurbitaceae	Bryonia	alba	Acetate 03	-43				-62	-50
Cucurbitaceae	Citrullus	colonythilis	control 01					-24	
Cucurbitaceae	Citrullus	colonythilis	Acetate 03					-4	
Cucurbitaceae	Cucumis	sativus	control 01					-26	
Cucurbitaceae	Cucumis	sativus	Acetate 03					-13	
Cucurbitaceae	Lagenaria	siceraria	control 01					-21	
Cucurbitaceae	Lagenaria	siceraria	Acetate 03					-29	-46
Cucurbitaceae	Momordica	charantia	control 01						
Cucurbitaceae	Momordica	charantia	Acetate 03	-93				-91	-88
Cucurbitaceae	Trichosanthes	kiriowii	control 01						
Cucurbitaceae	Trichosanthes	kiriowii	Acetate 03				-65		
Ebenaceae	Diospyros	kaki	control 01						
Ebenaceae	Diospyros	kaki	Acetate 03	-30			-95	-98	
Fabaceae	Albizia	julibrissin	control 01						-1
Fabaceae	Albizia	julibrissin	Acetate 03					-62	-30
Fabaceae	Cytisus	albus	control 01						
Fabaceae	Cytisus	albus	Acetate 03	-18			-50		
Fabaceae	Cytisus	scoparius	control 01						
Fabaceae	Cytisus	scoparius	Acetate 03	-41			-96	-95	







Polemoniaceae	Lianthus	grandiflorus	Acetate 03				-10	-80
Polemoniaceae	Lianthus	grandiflorus	control 01	-36			-88	-87
Polemoniaceae	Lianthus	grandiflorus	Acetate 03				-79	-76
Polemoniaceae	Polemonium	caeruleum	control 01					
Polemoniaceae	Polemonium	caeruleum	Acetate 03					
Polemoniaceae	Polemonium	caeruleum	control 01	-84			-73	-80
Polemoniaceae	Polemonium	caeruleum	Acetate 03	-70			-52	
Polemoniaceae	Polygonum	odoratum	control 01					
Polygonaceae	Polygonum	odoratum	Acetate 03					-37
Polygonaceae	Polygonum	odoratum	control 01					-14
Polygonaceae	Polygonum	odoratum	Acetate 03					
Primulaceae	Lysimachia	punctata	control 01				-35	
Primulaceae	Lysimachia	punctata	Acetate 03	-79			-81	-86
Primulaceae	Primula	japonica	control 01					
Primulaceae	Primula	japonica	Acetate 03	-47	-74	-88		
Rosaceae	Agrimonia	pilosa	control 01					
Rosaceae	Agrimonia	pilosa	Acetate 03		-16			
Rosaceae	Filipendula	vulgaris	control 01					
Rosaceae	Filipendula	vulgaris	Acetate 03	-71	-52			
Rosaceae	Filipendula	vulgaris	control 01					
Rosaceae	Filipendula	vulgaris	Acetate 03					-43
Saxifragaceae	Bergenia	crassifolia	control 01					-19
Saxifragaceae	Bergenia	crassifolia	Acetate 03					
Scrophulariaceae	Collinsia	heterophylla	control 01					-62
Scrophulariaceae	Collinsia	heterophylla	Acetate 03					-84
Scrophulariaceae	Digitalis	grandiflora	control 01	-58			-88	-80
Scrophulariaceae	Digitalis	grandiflora	Acetate 03				-84	
Scrophulariaceae	Gratiola	officinalis	control 01			-30		
Scrophulariaceae	Gratiola	officinalis	Acetate 03			-51		
Thiaceae	Grewia	pachycalyx	control 01					-8
Thiaceae	Grewia	pachycalyx	Acetate 03					
Valerianaceae	Valerianella	locusta	control 01					
Valerianaceae	Valerianella	locusta	Acetate 03	-4				
Verbenaceae	Verbena	tenuisecta	control 01					
Verbenaceae	Verbena	tenuisecta	Acetate 03	-48			-46	-58

The data indicate that a substantial number of plants exhibit anti-cancer activity following elicitation with 0.1% (v/v) acetic acid.

Several of the many plant extracts subjected to the anti-microbial assay yielded extracts having no detectable anti-cancer activity, whether induced by acetic acid or not, such as the following: *Baphicacanthus cusia*, *Actinidia chinensis*, *Aloinopsis luckhoffii*, *Aloinopsis rubrolineata*, *Aloinopsis spathulata*, *Delosperma ashtonii*, *Ruschia indurata*, *Ruschia pulvinaris*, *Tetragonia decumbens*, *Allium ampeloprasum*, *Allium tuberosum*, *Achyranthes bidentata*, *Amaranthus retroflexus*, *Amaranthus tricolor*, *Celosia cristata*, *Malosma laurina*, *Schinus terebinthifolius*, *Asimina triloba*, *Ammi majus*, *Angelica polymorpha*, *Apium graveolens*, *Carum carvi*, *Carum copticum*, *Cnidium monnieri*, *Cryptotaenia japonica*, *Cuminum cyminum*, *Heracleum sphondylium*, *Hydrocotyle asiatica*, *Levisticum officinale*, *Ligusticum porteri*, *Petroselinum crispum*, *Pimpinella anisum*, *Saposhnikovia divaricata*, *Rauvolfia caffra*, *Monstera deliciosa*, *Zantedeschia albomaculata*, *Aralia racemosa*, *Fatsia japonica*, *Araucaria araucana*, *Phoenix reclinata*, *Protasparagus africanus*, *Bulbine aloides*, *Kniphofia baurii*, *Achillea ptarmica*, *Ageratum conyzoides*, *Antennaria dioica*, *Arctium lappa*, *Arnica chamissois*, *Artemisia absinthium*, *Artemisia apiacea*, *Aster novae-angliae*, *Carlina acaulis*, *Centaurea maculosa*, *Chrysanthemum leucanthemum*, *Cichorium endivia*, *Corethrogyne californica*, *Cosmos sulphureus*, *Cynara cardunculus*, *Cynara scolymus*, *Eclipta alba*, *Eupatorium cannabinum*, *Eupatorium maculatum*, *Helenium autumnale*, *Inula ensifolia*, *Inula helenium*, *Lactuca sativa*, *Leontopodium alpinum*, *Liatis spicata*, *Pyrethrum corymbosum*, *Ratibida columnifera*, *Rudbeckia hirta*, *Serratula tinctoria*, *Silphium laciniatum*, *Silybum marianum*, *Spilanthes oleracea*, *Tagetes minuta*, *Taraxacum officinale*, *Telekia speciosa*, *Tussilago farfara*, *Xanthium sibiricum*, *Xanthium strumarium*, *Impatiens balsamina*, *Begonia sutherlandii*, *Nandina domestica*, *Podophyllum emodii*, *Podophyllum hexandrum*, *Betula nigra*, *Betula pendula*, *Corylus avellana*, *Chilopsis linearis*, *Adansonia digitata*, *Anchusa officinalis*, *Borago officinalis*, *Cynoglossum firmament*, *Heliotropium arborescens*, *Lithospermum officinale*, *Symphytum officinalis*, *Brassica campestris*, *Brassica hirta*, *Brassica nigra*, *Brassica oleracea*, *Brassica rapa*, *Crambe aspera*, *Hesperis matronalis*, *Isatis tinctoria*, *Lepidium sativum*, *Lunaria annua*, *Nasturtium officinale*, *Raphanus sativus*, *Berzelia abrotanoides*, *Calycanthus floridus*, *Chimonanthus praecox*, *Adenophora bulleyana*,

Campanula carpatica, Campanula persicifolia, Campanula rapunculcus, Campanula trachelium, Codonopsis pilosula, Lobelia siphilitica, Platycodon grandiflorum, Platycodon grandiflorus, Humulus lupulus, Capparis inermis, Kolkwitzia amabilis, Lonicera chamosi, Lonicera maackii, Lonicera tatarica, Sambucus nigra, Sambucus tigranii, Carica papaya, Cerastium tomentosum, Dianthus gratianopolitanus, Lychnis alba, Lychnis coriacea, Stellaria media, Maytenus capitata, Chenopodium ambrosioides, Chenopodium botrys, Chenopodium nuttalliae, Cistus incanus, Cistus ladanifer, Fumana procumbens, Helianthemum grandiflorum, Hypericum androsaemum, Hypericum olympicum, Hypericum perforatum, Terminalia mantaly, Cyanotis speciosa, Convolvulus cantabrica, Ipomoea purpurea, Camptotheca acuminata, Nyssa aquatica, Sedum spurium, Sedum telephium, Cucumis metuliferus, Calocedrus decurrens, Cupressus lusitanica, Cupressus sempervirens, Thuja occidentalis, Thuja orientalis, Cladium mariscus, Cyperus esculentus, Scirpus californicus, Scirpus robustus, Dioscorea dregeana, Dipsacus fullonum, Dipsacus sativus, Knautia arvensis, Scabiosa caucasica, Scabiosa lucida, Succisa pratensis, Dracaena hookeriana, Diospyros virginiana, Elaeagnus commutata, Hippophaë rhamnoides, Ephedra nevadensis, Ephedra viridis, Eremocarpus setigerus, Euphorbia lathyrus, Securinega suffruticosa, Euptelea pleiosperma, Abrus precatorius, Acacia redolens, Acacia saligna, Albizia kolomikta, Astragalus membranaceus, Astragalus sinicus, Cassia hebecarpa, Cassia tora, Ceratonia siliqua, Chamaecrista fasciculata, Cicer arietinum, Dalea candida, Erythrina christa-galli, Erythrina corallodendron, Galega officinalis, Glycyrrhiza echinata (glabra?), Glycyrrhiza glabra, Glycyrrhiza glabra (echinata?), Indigofera suffruticosa, Indigofera tinctoria, Lablab purpureus, Laburnum anagyroides, Lespedeza capitata, Lupinus arboreus, Lupinus perennis, Lupinus propinquus, Medicago sativa, Ononis spinosa, Petalostemon candidum, Pterocarpus indicum, Pueraria lobata, Pueraria thunbergiana, Robinia pseudoacacia, Sophora japonica, Tamarindus indica, Tephrosia grandiflora, Thermopsis fabacea, Thermopsis montana, Wisteria sinensis, Castanea dentata, Dovyalis caffra, Centaurium littorale, Gentiana lutea, Gentiana macrophylla, Gentiana tibetica, Erodium cicutarium, Geranium maculatum, Geranium sibiricum, Ginkgo biloba, Globularia aphyllanthes, Globularia trichosanthos, Aesculus hippocastanum, Albuca altissima, Hosta fortunea, Lachenalia pusilla, Philadelphus incanus, Nemophila maculata, Nemophila menziesii, Phacelia bolanderi, Phacelia campanularia, Phacelia tanacetifolia, Belamcanda chinensis, Gladiolus cunonius, Iris

*germanica*, *Iris notha*, *Iris pseudoacorus*, *Iris sibirica*, *Iris versicolor*, *Juglans nigra*, *Juncus acutus*, *Acinos thymoides*, *Agastache mexicana*, *Agastache nepetoides*, *Ballota nigra*, *Calamintha nepeta*, *Clinopodium vulgare*, *Elsholtzia stauntonii*, *Glechoma hederacea*, *Hyssopus officinalis*, *Lavandula officinalis*, *Leonotis nepetifolia*, *Leonurus cardiaca*, *Leonurus sibiricus*, *Lycopus europeus*, *Marrubium incanum*, *Mentha spicata*, *Monarda citriodora*, *Monarda fistulosa*, *Nepeta cataria*, *Origanum hirtum*, *Perilla frutescens*, *Pogostemon patchouli*, *Prunella vulgaris*, *Pycnanthemum pycnanthemoides*, *Rosmarinus officinalis*, *Salvia apiana*, *Salvia mellifera*, *Salvia officinalis*, *Satureja hortensis*, *Schizonepeta tenuifolia*, *Scutellaria altissima*, *Scutellaria orientalis*, *Sideritis syriaca*, *Stachys byzantina*, *Teucrium chamaedrys*, *Thymus cretaceus*, *Thymus vulgaris*, *Lanaria lanata*, *Stauntonia hexaphylla*, *Cinnamomum camphora*, *Laurus nobilis*, *Lilium martagon*, *Limnathes douglasii*, *Linum grandiflorum*, *Linum hirsutum*, *Linum perenne*, *Kissenia capensis*, *Mentzelia lindleyi*, *Strychnos spinosa*, *Lagerstroemia indica*, *Liriodendron tulipifera*, *Magnolia grandiflora*, *Alcea rosea*, *Althaea broussonetifolia*, *Althaea cannabina*, *Althaea officinalis*, *Gossypium herbaceum*, *Hibiscus sabdariffa*, *Hibiscus trionum*, *Malacothamnus fasciculatus*, *Dissotis senegambiensis*, *Toona serrata*, *Toona sinensis*, *Montinia caryophyllaceae*, *Ficus religiosa*, *Morus nigra*, *Morina longifolia*, *Myrica cerifera*, *Melaleuca alternifolia*, *Myrtus communis*, *Abronia nana*, *Mirabilis jalapa*, *Forsythia suspensa*, *Fraxinus excelsior*, *Fraxinus pennsylvanica*, *Ligustrum sinense*, *Syringa amurensis*, *Syringa villosa*, *Syringa vulgaris*, *Camissonia cheiranthifolia*, *Camissonia pallida*, *Clarkia amoena*, *Clarkia rubicunda*, *Oenothera lamarckiana*, *Oenothera speciosa*, *Argemone mexicana*, *Papaver bracteatum*, *Papaver dubium*, *Papaver orientale*, *Passiflora edulis*, *Ceratotheca sesamoides*, *Dicerocaryum eriocarpum*, *Harpagophytum zeyheri*, *Sesamum indicum*, *Phytolacca americana*, *Cedrus deodara*, *Pinus pinea*, *Plantago asiatica*, *Plantago erecta*, *Plantago hirtella*, *Plantago insularis*, *Plantago major*, *Plantago sempervirens*, *Plantago squalida*, *Platanus occidentalis*, *Armeria juniperifolia*, *Limonium gmelinii*, *Bouteloua curtipendula*, *Cortaderia selloana*, *Cymbopogon flexuosus*, *Panicum virgatum*, *Polypogon monspeliensis*, *Schizachyrium scoparium*, *Setaria sphacelata*, *Sorghum sudanense*, *Vetiveria zizanioides*, *Podocarpus falcatus*, *Gilia capitata*, *Gilia tricolor*, *Phlox drummondii*, *Phlox paniculata*, *Securidaca longepedunculata*, *Polygonum hydropiper*, *Polygonum lapathifolium*, *Polygonum orientale*, *Polygonum punctatum*, *Rheum palmatum*, *Rumex acetosa*, *Rumex crispus*,

Calandraria ciliata, Claytonia perfoliata, Portulacca oleracea, Anagallis arvensis, Primula elatior, Grevillea robusta, Punica granatum, Aconitum napellus, Adonis aestivalis, Aquilegia transsilvanica, Aquilegia vulgaris, Clematis manschurica, Clematis virginiana, Delphinium ajacis, Nigella arvensis, Nigella damascena, Nigella sativa, Thalictrum flavum, Trollius europaeus, Reseda luteola, Reseda odorata, Ceanothus cuneatus, Paliurus spina-christi, Rhamnus cathartica, Agrimonia eupatoria, Aruncus dioicus, Aruncus kamtschaticus, Cercocarpus betuloides, Chaenomeles japonica, Geum faurieri, Geum urbanum, Holodiscus discolor, Pentaphylloides mandschurica, Potentilla recta, Potentilla tormentilla, Prinsepia sinensis, Sanguisorba officinalis, Galium spurium, Gardenia jasminoides, Rubia tinctorum, Phellodendron amurense, Cardiospermum grandiflorum, Cardiospermum halicacabum, Koelreuteria paniculata, Sideroxylon inerme, Anemopsis californica, Bergenia cordifolia, Heuchera pilosissima, Tellima grandiflora, Schisandra chinensis, Antirrhinum majus-maximum, Collinsia canadensis, Hebenstreitia fastigiosa, Linaria vulgaris, Mimulus guttatus, Penstemon barbatus, Scrophularia nodosa, Vernonia noveboracensis, Veronica spicata, Simmondsia chinensis, Atropa bella-donna, Brugmansia suaveolens, Capsicum chinense, Datura stramonium, Hyoscyamus niger, Lycium barbatum, Nicandra physaloides, Physalis ixocarpa, Solanum aculeatissimum, Solanum dulcamara, Withania somnifera, Brachychiton discolor, Firmiana simplex, Fremontodendron californicum, Sterculia africana, Sterculia quinqueloba, Strelitzia nicolae, Strelitzia reginae, Cryptomeria japonica, Cunninghamia lanceolata, Taxodium distichum, Camellia japonica, Camellia sinensis, Grewia biloba, Grewia flavescens, Tropaeolum majus, Celtis occidentalis, Ulmus americana, Urtica dioica, Caryopteris sp., Verbena officinalis, Vitex agnus-castus, Cyphostemma juttae, Vitis amurensis, Vitis labrusca, Welwitschia mirabilis, Elettaria cardamomum, Larrea tridentata, Peganum harmala, and Tribulus terrestris.

In addition, the following plants responded to acetic acid by yielding extracts that inhibited an anti-cancer activity observed in the extracts of un-elicited plants: *Beaucarnea recurvata*, *Cotinus coggygia*, *Eryngium campestre*, *Aristolochia clematis*, *Cnicus benedictus*, *Grindelia robusta*, *Hieracium pilosella*, *Dianthus barbatus*, *Saponaria officinalis*, *Euonymus koopmannii*, *Luffa aegyptiaca*, *Dioscorea batatas*, *Elaeagnus angustifolia*, *Trigonella foenum-graecum*, *Psidium guajava*, *Eschscholzia caespitosa*, *Consolida orientalis*, *Mimulus puniceus*, *Ailanthus altissima*, and *Valeriana officinalis*.

